

# THE PROTRACTILE APPARATUS OF THE MOUTH OF THE PUMPKINSEED SUNFISH, EUPOMOTIS GIBBOSUS L.

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SEVEN FIGURES

Jaws that shut with a swinging action, like the grasping portion of pincers, are so common that the word 'jaw' suggests pincer-like action. However, there are several kinds of fish which, although retaining the swinging mandible, have in addition a complicated upper jaw which is protrusive in a horizontal or an oblique direction (fig. 1). The action of this upper jaw is simultaneous with that of the lower. Under ordinary conditions, protrusions are practically instantaneous—barely within the limit of vision. One can feel the distinct jerk of protraction and can hear the accompanying snap of retraction if the fish is held in the hand out of water. The premaxillae in these forms, being the parts that are thrust out, are said to be protractile. The maxillae are not fused with the premaxillae, although the former on either side are attached to the latter and serve as levers to protract them. The resulting instantaneous movement is of great advantage to the fish.

A fish having a protractile apparatus has the same advantage as a soldier with a bayonet six inches longer than that of his opponent. When the soldier comes to 'long point,' he outreaches his opponent by six inches, other things being equal. Likewise, a fish is brought nearer to its prey by just its range of protraction. Thus another fish, swimming at the same rate, but with ordinary jaws, might lose its intended

victim only by this small margin. A fish with protractile premaxillaries has the advantage in surprising its victim. The shadow of a vertically closing, open jaw is ample enough warning for some of the quick-moving, small creatures of lake waters to escape; but a mouth that can be shot out horizontally over its victim gives no hint of danger. A bluegill (*Lepomis pallidus*) or pumpkinseed sunfish (*Eupomotis gibbosus*) can nibble at bait, and usually get it, where a bullhead (*Ameiurus*) would be caught, because the first two have protractile upper jaws. They do not bite down upon the hook. They snap their mouths out horizontally over the hook and withdraw them the same way, probably in so doing raking the bait along with them. The fisherman feels this snap as a nibble on his line, and if he pulls just when the snap occurs, he will hook the fish through the roof of the mouth, usually between premaxilla and maxilla. In feeding, by means of this apparatus a fish can extend its range of food to flies, gnats, and other insects which hover near the surface of the water, to such snails as live on the rocks of the shoreline where the waves wash over them, or to floating objects, such as grasshoppers which have accidentally jumped out over the water. It is much more efficient, both in regard to energy expended and to certainty of catch, for a fish to snap its extensible mouth above water to catch an unwary fly than for it to jump above the surface to catch it. I have seen fish with protractile jaws thus catch flies, and have also found the remains of flies in the stomach. While I was walking through the tall grass bordering the canals south of Miller's Bay, West Okoboji Lake, many grasshoppers, frightened from their stalks of grass, would jump into the canal; but, if the day were sunny, these would hardly touch the water before being snapped up by sunfish.

The efficiency of fish in catching insects flying near the surface is of economic importance. It increases the range of food for game fish, directly in the case of perch (*Perca flavescens*), black bass (*Micropterus salmoides*), and those other large game fish possessing protractile upper jaws, and



indirectly in the case of sunfish and minnows, many of which have protractile upper jaws, as these form a bulk of the food of carnivorous game fish.

While protractile premaxillaries have been used extensively by the zoölogist in classifying fish, the mechanism of protraction and retraction has been left relatively unstudied, especially in regard to fresh-water fish. Owen<sup>1</sup> has worked out the myology of the head of the perch (*Perca fluviatilis*), but has explained the protraction and retraction of the pre-

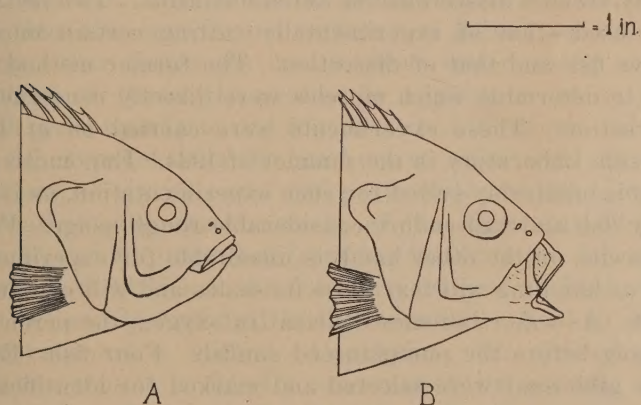


Fig. 1.

Fig. 1 Head of *Eupomotis gibbosus*, the pumpkinseed sunfish. A. Premaxillae retracted. B. Premaxillae protracted.

maxillaries only in a very general way. Gunther<sup>2</sup> gives the osteology of the perch, but likewise gives very little detail in regard to the bones of the protractile apparatus. Owen,<sup>3</sup> however, has described, in some detail, the bones of the protractile apparatus of one of the wrasses, *Epibulus insidiator*, which he compares with like structures in other wrasses. The musculature of the mechanism is not given. The protractile apparatus in *Epibulus*, as he gives it, is more highly developed

<sup>1</sup> Owen. *Anatomy of Vertebrates*, vol. 1, pp. 205-209.

<sup>2</sup> Gunther. *The Study of Fishes*, pp. 51-62.

<sup>3</sup> Owen. *Anatomy of Vertebrates*, p. 119.

than that of *Eupomotis gibbosus*, described in this article. *Epibulus* differs chiefly in having a longer pedicle to the premaxillae (the intermaxillaries of Owen), and in having a movable quadrate bone (the hypotympanic of Owen), by means of which the lower jaw is also protracted.

In studying the mechanism of the protractile jaws, the pumpkinseed sunfish (*Eupomotis gibbosus* Linnaeus) was taken as typical. Comparisons were made with dissections of the perch (*Perca flavescens* Mitchill) which followed closely Owen's dissections on *Perca fluviatilis*. Two methods were used—that of experimentally cutting certain muscles on live fish and that of dissection. The former method was used to determine which muscles were directly concerned in protraction. These experiments were carried on at Iowa Lakeside Laboratory in the summer of 1924. *Eupomotis gibbosus* is admirably suited for such experimentation, as it is a hardy fish and will endure considerable rough usage. *Perca flavescens*, on the other hand, is unsuitable for experimentation, as handling will tear loose its scales and will eventually kill it. As water becomes deficient in oxygen, the perch will die long before the pumpkinseed sunfish. Four fish (*Eupomotis gibbosus*) were selected and marked for identification as nos. 43, 44, 45, and 46. The fish thrust out the premaxillae as the oxygen content of the water decreased. If the oxygen content was greatly deficient, protraction was not of the quick, instantaneous type, but more slow, often prolonged. This method of compelling protraction proved too long for experimental purposes, so a better one was devised. This consisted simply in holding the fish in the hand out of water for a few moments. In normal fish this would evoke a series of rapid, normal protractions. The former method was used for the beginning experiments, later the other was substituted.

By observation the paired geniohyoideus<sup>4</sup> seemed to be the chief factor in protraction. When it contracted the mandible was opened, causing the unpaired intermandibularis<sup>5</sup> muscle

<sup>4</sup> See figure 7.

<sup>5</sup> See figures 6 and 7.



to pull the ventral ends of the maxillae<sup>6</sup> forward, which in turn thrust the premaxillae forward (figs. 2, 3, 4, 5). To determine whether the geniohyoideus was the chief factor in protraction, this muscle on both sides was severed on nos. 43 and 44, but left intact on nos. 45 and 46. The fish were then returned to the aquarium and observed from 10.30 A.M. until 12.25 P.M. From 11 o'clock to 11.45 nos. 45 and 46, uninjured, were gulping at the surface for air, thrusting out the protractile apparatus with increasing frequency to half its full extent. Nos. 43 and 44, geniohyoideus severed, during this time also came to the surface and gulped air, but in so doing either thrust the mouth above water, using no protraction, or thrust the protractile apparatus out by short, quick jerks usually to one-fourth of its full extent. At 11.43 the fish were taken in the hand out of water with the result that nos. 43 and 44 could still protract to one-third, or even one-half, of the extreme range of the apparatus by jerks. Nos. 45 and 46 protracted from two-thirds to the full extent, slowly. At 12.25 P.M. the fish were changed to fresh water. Nos. 43 and 44 had died in the meantime. Nos. 45 and 46 soon returned to normal. Conclusion: The paired geniohyoideus muscle is directly concerned in the extreme extension of both jaws and in protractions lasting over one-half second in time. Short, instantaneous protractions are due to some other factor.

The paired frontomaxillaris muscle was similarly tested on specimen no. 48 (*Eupomotis gibbosus*). The frontomaxillaris attaches to the dorsal end of the maxilla and pulls this part of it posteriorly. The bone acts as a lever on its fulcrum—the process of the lateral ethmoid.<sup>7</sup> The lower part of the maxilla on each side is thrust forward, forcing the ventral part of the premaxilla ahead of it, thus causing protraction. This muscle on both sides was cut, and the fish held out of the water at intervals. It was able to thrust the premaxillae slowly forward. Some jerking of the head caused a slight protraction of the premaxillae. Conclusion: The paired

<sup>6</sup> See figures 2, 3, 4, and 5.

<sup>7</sup> See figures 3, 4, and 5.

frontomaxillaris muscle is the chief element in quick protraction. The geniohyoideus is confirmed as the agent of slow, extended protraction. Other factors may cause protraction to a certain degree.

The geniohyoideus on both sides on no. 48 was then severed. The protractile apparatus at first seemed to hang loosely, but with apparent effort was slowly extended. Conclusion: Some other element than the geniohyoideus and the frontomaxillaris is inefficiently capable of protracting the jaw. When the thumbs were placed on the cheeks of the fish, the protractile apparatus was forced out in the same way. Therefore, it seems that the tightening of the skin over the pedicle of the premaxillae, caused by an approximation of the sides of the palatine arch, can with difficulty squeeze the pedicle of the premaxillae out of its groove. This movement is accompanied by many sharp, apparently fruitless jerks of the whole head in which the movement seems to be directed upward. The muscles probably involved in this action will be considered in the discussion of the dissection.

The dissection was carried out both at Iowa Lakeside Laboratory and at the Laboratories of Animal Biology of the State University of Iowa, most of the work being done at the latter place. During the summer of 1924 each specimen had been numbered when caught, and a record kept of the time, the place, the method used (seine or hook and line), and other data. Such data has its greatest value in ecological studies, as examination of stomach contents, but it has helped in these studies in selecting specimens which were least mutilated as to mouth-parts. The fish were preserved in 10 per cent formalin, and were washed in water and changed to 70 per cent alcohol just before dissection, to avoid the fumes of the formalin. Most of the work was gross dissection, although a hand lens was found necessary at times. In the study of the bones the fish were boiled in a solution of 'Gold Dust' and water to loosen the flesh previous to dissection.

The bones immediately concerned in the protractile action of the upper jaw are the lateral ethmoids, maxillae, ethmoid,



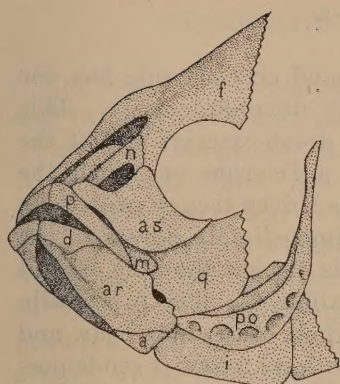


Fig. 2.

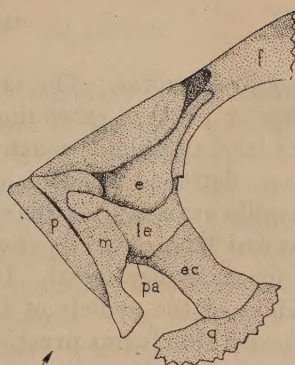


Fig. 3.

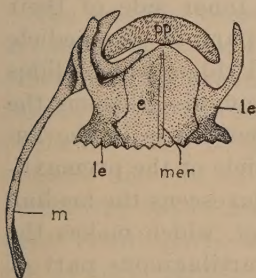


Fig. 4.

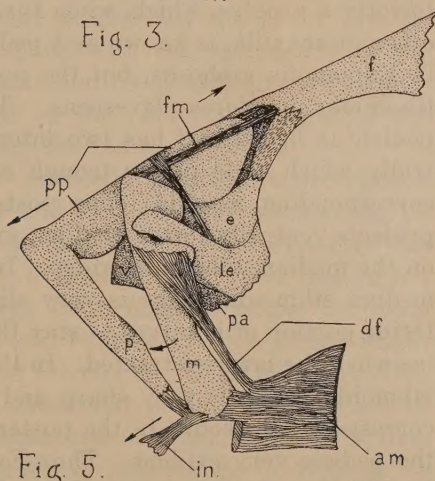


Fig. 5.

Fig. 2 Bones of the anterior head region, *Eupomotis gibbosus*. Superficial dissection. *a*, angular; *ar*, articular; *as*, anterior suborbital; *d*, dentary; *f*, frontal; *i*, interopercle; *m*, maxilla; *n*, nasal; *p*, premaxilla; *po*, preopercle; *q*, quadrate.

Fig. 3 Lateral view of a deeper dissection of bones of preorbital region of head, *Eupomotis gibbosus*. *e*, ethmoid; *ec*, ectopterygoid; *f*, frontal; *le*, lateral ethmoid; *m*, maxilla; *p*, premaxilla; *pa*, palatine; *q*, quadrate.

Fig. 4 Posterior view of maxilla and related parts, *Eupomotis gibbosus*. *e*, ethmoid; *le*, lateral ethmoid; *m*, maxilla; *mer*, median ethmoidal ridge; *pp*, pedicle of the premaxillae—anterior part in cross-section.

Fig. 5 Lateral view of protractile premaxilla and related parts, *Eupomotis gibbosus*. Arrows indicate movement of muscles and bones necessary for protraction. Bones: *e*, ethmoid; *f*, frontal; *le*, lateral ethmoid; *m*, maxilla; *p*, premaxilla; *pa*, palatine; *pp*, pedicle of the premaxillae; *v*, vomer. Muscles: *am*, adductor mandibulae; *df*, dorsal fasciculus of adductor mandibulae; *fm*, frontomaxillaris; *in*, intermandibularis.

and premaxillae. The lateral ethmoid on each side has one slender process extending obliquely dorso-anteriorly. This fits into an oblique notch near the dorso-exterior end of the long, flattened maxilla, acting as a fulcrum on which the maxilla swings. The maxilla carries a deep trough projecting inward from near its dorsal end, immediately dorsal to the notch just mentioned. It is in this trough that the lateral ridges of the pedicle of the premaxillae fit. The premaxilla on each side forms practically the whole of the upper jaw and bears teeth. Near its articulation with its fellow it sends posteriorly a process, which, when fused with the process of the other premaxilla, is known as a pedicle. This pedicle is bony in *Eupomotis gibbosus*, but the posterior part remains cartilaginous in *Perca flavescens*. The anterior part of the pedicle is broad and has two lateral ridges projecting ventrally which slide in the trough on the inner side of their corresponding maxilla. The posterior part of the pedicle projects ventrally in the median, grooved ridge, which slides on the median, ethmoidal ridge. In *Eupomotis gibbosus* the median ethmoidal ridge is very slight, but a rise in the anterior portion of it serves to stay the pedicle of the premaxillae when the jaw is retracted. In *Perca flavescens* the median ethmoidal ridge is very sharp and abrupt, which makes the corresponding groove in the posterior, cartilaginous part of the pedicle very evident. Thus *Eupomotis gibbosus* has an interlocking device to stay the pedicle of the retracted premaxillae which the perch does not possess. This is probably one of the reasons why prolonged protraction occurs so easily and often in the perch and so seldom in the sunfish. The premaxillae and maxillae are the moving elements in this mechanism. In addition to the lateral ridges of the pedicle of the premaxillae sliding on the maxillae, each premaxilla at its ventral tip is connected by cartilaginous strands with the lower anterior margin of the maxilla. As the lower part of the latter swings anteriorly in acting upon its fulcrum (the process of the lateral ethmoid) protraction of the premaxilla is forced by this connection.



The lower jaw is composed of two dentary, two articular, and two angular bones, and a remnant of Meckel's cartilage which lies in the groove on the inner surface of each articular. All are closely fused. The dentary alone of these bones bears teeth. The two dentaries meet in an immovable joint in midline. The angular, a small flattened bone just below the articulation of the articular with the quadrate, is attached by cartilaginous strands to the interopercle. On the ventro-posterior margin of the articular is a transversely placed groove which articulates with a corresponding prominence on the quadrate. While not protractile, the lower jaw increases its reach to correspond with the protraction of the upper jaw by having this articulation of articular with quadrate quite ventrally placed on the posterior margin of the former bone, thus increasing the radius of action.

The muscles<sup>\*</sup> directly or indirectly involved in protraction are as given below (figs. 6 and 7). As some muscles act on other muscles causing them to act, and others back of the former might act on them, it is clear that, even though improbable, some of the large body muscles might act upon muscles anterior to them, and eventually such action might be transmitted to muscles of the mouth. Such indirect action upon mouth-parts is not considered in this article back of the pectoral girdle. The branchial-arch musculature is likewise not considered of sufficient importance in relation to protraction to be described. The only action it could have in protraction would be the raising of the opercle by extreme expansion of the branchial arches, which indirectly might cause some movement farther anterior. However, an equivalent effect would be secured if the opercle were raised by the muscles which ordinarily act upon it. As the muscles of *Eupomotis gibbosus* are shown in the figures as drawn to scale, measurements will be omitted from the description.

*Adductor mandibulae.* This is the largest muscle in the head. With its fellow it forms the cheeks of the fish. It has an extensive origin, most of its fibers arising from a groove

<sup>\*</sup> See figures 6 and 7.

along the entire anterior border of the preopercle, excepting the dorsal portion of the extreme anterior part. Some of its fibers arise from the lower part of the hyomandibular. This muscle is divided into a cephalic and a mandibular portion. Many fibers of the mandibular portion arise from the anterior border of the quadrate near its articulation with the lower jaw and continue forward with the others. In addition to the above, a bundle of fibers arises on the dorsoposterior part of the metapterygoid under the insertion of the levator arcus palatini. The insertion is, 1) by a slender dorsal fasciculus into the posterior and outer border of the maxilla at a point just ventral to its articulation with the process of the lateral ethmoid; 2) by a fusion of fibers with the membrane binding the bones of the jaw together near the angle of the mouth, and, 3) into the posterior part of the articular bone dorsal to its articulation with the quadrate, into the outer posterior part of the dentary, and to the inner surface of the dentary just anterior to its junction with the articular. The band of muscle fibers thus attaching to the inner surface of the dentary lies just interior to Meckel's cartilage. The fibers of the adductor mandibulae run horizontally for the most part, although they converge from a fan-shaped origin on the preopercle. The muscle is more or less divided into two sheets—an exterior and an interior. The adductor mandibulae closes the mouth. Insertion (1) by swinging the maxillae on its fulcrum (the process of the lateral ethmoid) draws the ventral portion of that bone posteriorly. This action is helped by insertion (2) which pulls the angle of the jaw posteriorly. This closes the upper jaw, since the ventral parts of the premaxillae are connected, as previously described, with the ventral parts of the maxillae. Insertions (2) and (3) close the lower jaw.

*Geniohyoideus.* This broad, flat muscle lies ventral to the mouth cavity at one side of the midline and antagonizes the adductor mandibulae. It is fused with its fellow in midline as it runs anteriorly. This is more noticeable in the perch than in this fish. The muscle arises in an oblique line extend-



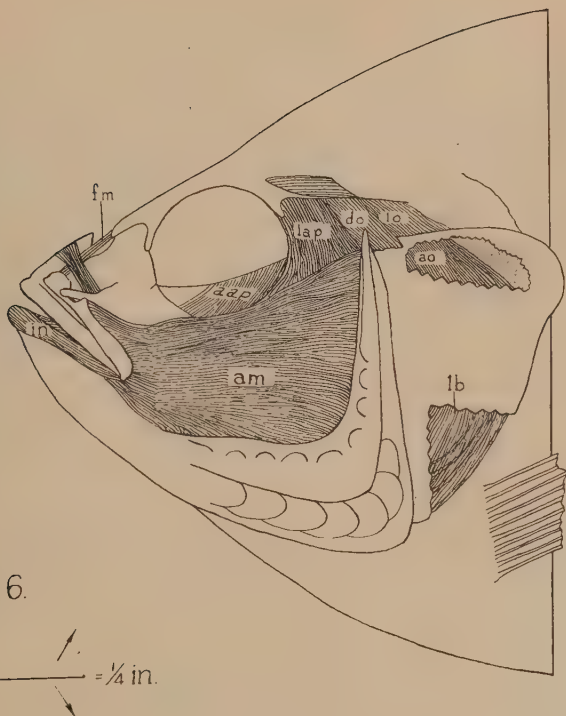


Fig 6.

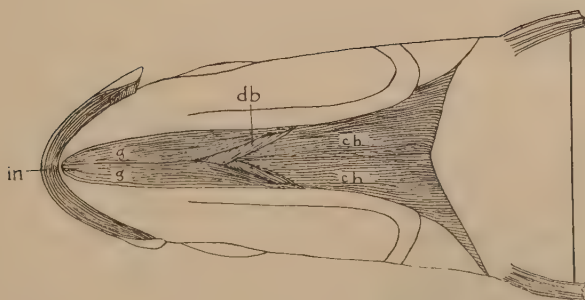


Fig. 7.

Fig. 6 Lateral view of musculature of the head, *Eupomotis gibbosus*. *aap*, adductor arcus palatini; *am*, adductor mandibulae; *ao*, adductor operculi; *fm*, fronto-maxillaris; *in*, intermandibularis; *lap*, levator arcus palatini; *lb*, levator branchiostegorum; *lo*, levator operculi.

Fig. 7 Ventral view of musculature of the head, *Eupomotis gibbosus*. *ch*, cleithrohyoideus; *db*, depressor branchiostegorum; *g*, geniohyoideus; *in*, intermandibularis.

ing from the posteroventral to the anterodorsal portions of the ceratohyal. Insertion is on the lower anterior portion of the dentary near the symphysis of the jaws. Greene in king salmon found this part of the muscle divided into an external and an internal portion, one passing above, the other below the intermandibularis to its insertion. This arrangement is more evident in perch than in this fish. The fibers of this muscle run longitudinally until under the base of the tongue where they separate in Y-fashion, each branch going obliquely to its insertion. The contraction of this muscle opens the lower jaw. By action of the intermandibularis (as explained under that muscle), it also serves to protract the upper jaw. Extreme contraction of this muscle in death in the perch aids the depressor branchiostegorum in opening out the branchiostegal rays to their greatest extent by pulling the ceratohyal ventrally and anteriorly. It is very common for the perch thus to die with mouth open and branchiostegal rays extended.

*Intermandibularis.* This is an unpaired muscle lying just outside and ventral to the margin of the lower jaw. It is covered with a thick layer of skin. It extends from the ventral end of one maxilla around to the ventral end of the other maxilla. This portion of it is approximately circular in cross-section at the symphysis of the lower jaw. Along the dorsal border of this portion it is attached on each side by a thin sheet of muscle to the dorsal margin of each dentary. The connection between the ventral tips of the premaxillae and the corresponding points of their respective maxillae causes the protraction of the premaxillae when the lower ends of the maxillae are pulled anteriorly by this muscle. The fibers of the intermandibularis run parallel with the margin of the jaw except in the thin, dorsal, sheet-like portion where they run dorsally and obliquely posterior. Its purpose in some fishes is to approximate the two dentaries, in which it would be antagonized by certain portions of the adductor mandibulae, levator arcus palatini, and to a lesser extent by the dilator operculi. However, in pumpkinseed sunfish it is modi-



fied to act as an intermediary of geniohyoideus to protract the upper jaw as described above. In this fish the connection of the intermandibularis with the maxillae is so noticeable that, aside from comparative reasons to the contrary, the name 'intermaxillaris' would appear more fitting than intermandibularis. In opening the protractile apparatus it is antagonized by the adductor mandibulae.

*Frontomaxillaris.* This is a paired muscle having its origin on the anterior margin of the frontal bone. It is a very slender slip of fibers. Its insertion is on the outer part of the dorsal projection of the maxilla. The purpose of this muscle is to protract the upper jaw. It does this by pulling the dorsal part of the maxilla posteriorly. As the maxilla swings on the process of the lateral ethmoid as its fulcrum, this pushes the lower portion of the maxilla forward, protracting the premaxilla on that side. In this action it is structurally opposed by the dorsal fasciculus of the adductor mandibulae, though in actual use it is probably opposed by the whole of the massive adductor mandibulae and aided by the geniohyoideus acting through the intermandibularis as previously described. When isolated, as described in experimenting on the living fish, it gives the instantaneous jerks to the jaw. Therefore, the quick starting of the act of protraction is probably its chief function.

There is a band of tough fibers on each side of the pedicle of the premaxillae which has its origin on the dorsal margin of the base of the process of the lateral ethmoid. Its insertion is in the middle part of the dorsal midline of the pedicle of the premaxillae. Its purpose is to hold the pedicle of the premaxillae down on the surfaces over which it slides. The frontomaxillaris on each side runs under this band of fibers.

The protractile apparatus may be affected indirectly by another muscle pulling on the movable origin of one of the above. Indeed, this we have seen to be the case in the action of the geniohyoideus upon the intermandibularis. This is unlikely to happen in other cases, excepting one—the possible action of the cleithrohyoideus on the bones forming the origin of the geniohyoideus.

*Cleithrohyoideus* (*sternohyoideus* of Greene), a paired muscle posterior to the geniohyoideus with its origin (considered according to its major function) along the basibranchiostegal extending on to the basihyal in the perch, but confined to the sides of the basibranchiostegal in the pumpkinseed sunfish. The small slip of muscle fibers on each side, which connects its anterior end with the basihyals in perch, is also present in the pumpkinseed sunfish, but totally disconnected from the anterior end of the cleithrohyoideus. A considerable stretch of the basibranchiostegal bone intervenes between the anterior end of the cleithrohyoideus muscle and the origin of this paired slip in the pumpkinseed sunfish. The cleithrohyoideus is inserted on the ventro-anterior end of the cleithrum. The major function of the cleithrohyoideus is to move the cleithrum forward. In this it is opposed by some muscle or sets of muscles farther posterior in the body. However, if the cleithrum remains stationary, the contraction of this muscle, acting on the origin of the geniohyoideus, may help to open the lower jaw. In this respect it would seem more probable of acting in the perch than in this sunfish, because of the more direct connection with the basihyals in the former, and because its fibers in the former run more longitudinally, i.e., not so obliquely toward midline as in the latter.

In a previous place in this article it was observed that a tightening of the skin over the pedicle of the premaxillae caused by an approximation of the sides of the palatine arch, might with difficulty squeeze the pedicle out of its groove, thus causing protraction. If so, the paired muscle concerned would be—

*Levator arcus palatini*, which fills the space posterior to the eyeball. It has its origin on the sphenotic and its insertion on the upper part of the metapterygoid and on the hyomandibular at the same level. Its chief purpose, other than the possible one noted above, is the dilation of the pharynx. It is opposed by—

*Adductor arcus palatini*, which arises on the prootic and parasphenoid and is inserted on the dorsal part of the inner



surface of the metapterygoid and on the inner surface of the hyomandibular at a slightly more dorsal level.

Other paired muscles controlling parts attached to the palatine arch might help or hinder the above muscles. These would be—

*Dilator operculi*, a muscle lying posterior to the levator arcus palatini and whose fibers are very difficult to separate from it. The fibers of the dilator operculi run past and partly under the dorsal end of the preopercle. This muscle arises from the anterior external border of the pterotic and posterior external border of the sphenotic. It is inserted in the dorso-anterior portion of the opercle. It moves the opercle outward, away from midline. It is opposed by—

*Adductor operculi*, arising from the ventral surface of the posterior part of the pterotic and squamosal and inserted into the upper margin of the ridge on the interior surface of the opercle. This ridge is a continuation of the base of the opercular spine in the perch. The ridge alone is present in the pumpkinseed sunfish.

*Levator operculi* arises from the posterior part of the ridge of the pterotic and is inserted into the anterior half of the dorsal margin of the opercle. It elevates the opercle. Not only might this affect the palatine arch, but it can also, by the connection of the opercle, subopercle, interopercle, angular, articular, and dentary bones, move the lower jaw. Such a movement, while possible, would be very indirect.

*Levator branchiostegorum*. This muscle arises along the ventral margin of the opercle in perch and along the posterior part of the ventral margin of the opercle in this sunfish. The origin is continued onto the dorsoposterior part of the subopercle in both fish. It is inserted in turn upon the inner margin of each branchiostegal ray. It elevates the branchiostegal rays. The opposing muscle is—

*Depressor branchiostegorum*, arising on the basihyal and anterior end of the ceratohyal and inserted on the ventral portion of the lowest branchiostegal ray. It is also attached to the ceratohyal and epihyal. This muscle has its origin

near the midline, but slightly to the opposite side from its insertion. In running obliquely back to its insertion, the left muscle passes obliquely ventral to its fellow near their origin.

#### SUMMARY

1. The bony apparatus of protraction in *Eupomotis gibbosus*, the pumpkinseed sunfish, consists of protractile premaxillae, the pedicles of which, partially supported by an inner process of the maxilla on each side, slides on the ethmoid. Each maxilla swings on the process of the lateral ethmoid of that side. The lower part of the maxillae forces the protraction of the premaxillae. The mandible is not protractile, although its action causes protraction in the upper jaw.

2. By muscular mechanism, protraction in the perch and this sunfish can be brought about in at least three ways: *a*) By action of the frontomaxillaris muscle—apparently for short, instantaneous protractions or in initiating protraction. *b*) By action of the geniohyoideus muscle on the intermandibularis. This action may start with the cleithrohyoideus. *c*) By indirect action of muscles farther back in the head.

3. The intermandibularis muscle, in addition to approximating the two sides of the mandible, is also used in carrying forward the lower ends of the maxillae in certain species of fish having protractile jaws.

4. Retraction is brought about by the adductor mandibulae muscle.

5. The easily provoked protraction of the perch, as compared with the usual retraction of the pumpkinseed sunfish, has a morphological foundation which lies, in part, in the shape of the ethmoid bone.

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## ADDENDA

For the convenience of those using these studies in connection with literature from generally recognized authorities in Ichthyology, the following tables are added. Only the terms herein used are given. For more extensive tables, consult synonymies listed in the bibliography.



*Synonymy of bones*

| TERM USED IN THESE STUDIES | OWEN           | OUVIER                                                    | PARKER             | OTHERS                                                       |
|----------------------------|----------------|-----------------------------------------------------------|--------------------|--------------------------------------------------------------|
| Angular                    | Angular        | Angulaire                                                 | Angular            | Articular (Stannius)                                         |
| Articular                  | Articulary     | Articulaire                                               | Articular          | Hypobranchial plate (Greene)                                 |
| Basibranchiostegal         | Urohyal        | Queue de l'os hyoïde                                      | Basibranchiostegal |                                                              |
| Basihyal                   | Basihyal       | Petite piece lateral                                      | Basihyal           |                                                              |
| Branchiostegal             | Branchiostegal | Rayons branchiosteges                                     | Branchiostegal     |                                                              |
| Ceratohyal                 | Ceratohyal     | Grande piece lateral (not differentiated from epihyal)    | Ceratohyal         |                                                              |
| Cleithrum                  | Coracoid       | Humeral                                                   | Clavicle           | Proscapular (Gill),<br>Cleithrum (Gegenbaur)                 |
| Dentary                    | Dentary        | Dentaire                                                  |                    |                                                              |
| Ectopterygoid              | Pterygoid      | Transverse                                                | Pterygoid          | Ectopterygoid (Huxley)                                       |
| Epihyal                    | Epihyal        | Grande piece lateral (not differentiated from ceratohyal) | Epihyal            |                                                              |
| Ethmoid                    | Nasal          | Ethmoid                                                   | Ethmoid            | Nasal (Vogt and Yung),<br>Ethmoid (Gunther)                  |
| Frontal                    | Frontal        | Frontal principal                                         | Frontal            | Temporal (Stannius),<br>Hyomandibular (Gunther)              |
| Hyomandibular              | Epi-tympanic   | Temporal                                                  | Hyomandibular      |                                                              |
| Interopercle               | Interoperculum | Inter-opercule                                            |                    |                                                              |
| Lateral ethmoid            | Prefrontal     | Frontal antérieur                                         | Lateral ethmoid    |                                                              |
| Maxilla                    | Maxillary      | Maxillare superieur                                       | Maxillary          |                                                              |
| Mesopterygoid              | Entopterygoid  | Pterygoidien interne                                      | Mesopterygoid      | Os tympanicum (Stannius),<br>Metapterygoid (Huxley, Gunther) |
| Metapterygoid              | Pretympanic    | Tympanal                                                  | Metapterygoid      |                                                              |
| Nasal                      | Turbinal       | Nasal                                                     | Nasal              | Nasal (Gill), Os terminale (Stannius)                        |
| Opercle                    | Operculum      | Operculaire                                               |                    |                                                              |
| Parasphenoid               | Basisphenoid   | Sphenoïde                                                 | Parasphenoid       |                                                              |

| Premaxilla | Intermaxillary or Premaxillary | Intermaxillary (Stannius)                        |
|------------|--------------------------------|--------------------------------------------------|
| Preopercle | Preoperculaire                 |                                                  |
| Prootic    | Grande aile du sphénoïde       | Prooticum (Huxley),<br>Ala temporales (Stannius) |
| Pterotic   | Mastoidien                     | Opisthoticum (Huxley)                            |
| Quadrate   | Jugal                          | Opisthoticum and squamosal (Huxley)              |
| Sphenotic  | Frontal postérieur             | Quadrate-jugal (Stannius)                        |
| Squamosal  | (See Pterotic)                 | Postfrontal (Gill)                               |
| Subopercle | Sous-opercule                  | Squamosal (Huxley)                               |
| Suborbital | Infraorbital ring              |                                                  |
| Vomer      | Vomer                          |                                                  |

*Synonymy of muscles*

| TERM USED IN THESE STUDIES | GREENE                  | OWEN                       |
|----------------------------|-------------------------|----------------------------|
| Adductor arcus palatini    | Adductor arcus palatini | Depressor tympani          |
| Adductor mandibulae        | Adductor mandibulae     | Retractor oris             |
| Adductor operculi          | Adductor operculi       | Depressor operculi         |
| Cleithrohyoideus           | Sterno-hyoideus         | Retractor hyoidei          |
| Depressor branchiostegorum | Hyo-hyoideus            | Depressor branchiostegorum |
| Dilator operculi           | Dilator operculi        |                            |
| Frontomaxillaris           |                         |                            |
| Geniohyoideus              | Geniohyoideus           | Geniohyoideus (of Cuvier)  |
| Intermandibularis          | Intermandibularis       | Intermandibularis          |
| Levator arcus palatini     | Levator arcus palatini  | Levator tympani            |
| Levator branchiostegorum   |                         | Levator branchiostegorum   |
| Levator operculi           | Levator operculi        | Levator operculi           |





## A RARE VARIATION IN THE PECTORALIS MINOR MUSCLE

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### TWO FIGURES

A very interesting and rare variation in the pectoral musculature appeared recently in our laboratory in the case of a pectoralis minor muscle which presented a complete insertion into the middle portion of the inferior surface of the clavicle.

The subject in which this anomalous insertion occurred was a negro woman about sixty-five years of age, and, with the exception of the variations in the insertions of the two minor pectoral muscles to be described, the musculature of the pectoral region was normal. The pectoralis major muscles presented no deficiencies—in fact, the major pectorals were unusually well developed for a female subject and the subclavius muscles also were normal both in size, form, and position.

The principal variation, and the one which is most worthy of note, was in the insertion of the pectoralis minor on the left side. This muscle arose from the third and fourth ribs and the fascia over the second and third interspaces. Passing freely over the second rib, its fibers were attached to the inferior surface of the clavicle in about its middle portion. Some of the fibers were also attached to the costocoracoid ligament, which presented an unusual degree of development, the ligament being almost tendinous in character. Some of the medial fibers of the pectoralis minor were found to intermingle with the lateral fibers of the subclavius muscle—a fact which we are inclined to regard as adventitious rather

than as indicative of a closer relation between the two muscles than appears under normal conditions. The fibers which form the insertion do not bear the same intimate relation to the bone which is seen in muscles that are constantly inserted into tuberosities, ridges, etc., but appear to follow the spread of the costocoracoid membrane over the inferior aspect of the clavicle.

The pectoralis minor on the right side corresponded to the muscle on the left in respect to origin and size, but differed in regard to its insertion. The fibers of this muscle were directed upward and laterally, a few fibers being attached to the lateral border of the coracoid process; the greater part of the insertion, however, was into the tendon of common origin of the biceps brachii and coracobrachialis muscles. Both muscles were supplied by the medial anterior thoracic nerve; the principal blood supply being by a branch of the thoraco-acromial artery which accompanied the nerve.

In view of the considerable literature which has been devoted to variations in the muscles of the pectoral group, relatively little has been said about the pectoralis minor. Those which have been reported by Le Double(7), who gives the most extensive account, consist mainly of insertions which pass beneath the coraco-acromial ligament to the capsule of the shoulder-joint, or which are attached to the common tendon of the biceps (short head) and the coracobrachialis, or which find their way to the greater tuberosity of the humerus in close association with the insertion of the supraspinatus muscle. A few cases are recorded in which a slip, evidently belonging to the pectoralis minor, is inserted into the inferior surface of the clavicle.

The occurrence of slips which pass to the inferior surface of the clavicle is rare. Taylor(8) mentions such a slip, the fibers of which arise from the second rib. Wood(9) describes two cases, one a small muscular slip, the other a tendinous fascicle, both arising from the second rib, their insertion being into the costocoracoid ligament and the inferior surface of the clavicle. Chudzinski (Le Double and Taylor)

also describes a strong muscular slip which arises from the second rib and is attached to a well-developed costocoracoid membrane.

Cunningham(3), Huntington(5, 6), and Adachi(1, 2) have made extensive reports on variations in the pectoral musculature. They make no mention, however, of a pectoralis minor having its entire insertion into the inferior surface of the clavicle.

In the case which is the subject of this report the insertion of the muscle on the right side corresponds to variations which have been recorded by Le Double. On the left side the insertion of the pectoralis minor into the costocoracoid ligament and inferior surface of the clavicle is not partial, but complete, there being no indication whatsoever of fibers by means of which an attachment to the coracoid process is secured.

This case also differs from those which have been hitherto reported in the fact that it receives no additional fibers from the second rib, above which it passes freely; hence it is quite unlikely to represent, in any portion, a slip which has been acquired from the subclavius. It is possible to regard slips which arise from the second rib and are inserted into the inferior surface of the clavicle as being derived from the subclavius muscle, although the view which holds that they belong to the pectoralis minor is probably correct.

The question whether variations of this kind are reversal or fortuitous is difficult to answer. Hepburn(4) describes the insertion of the pectoralis minor in the gibbon as consisting of three parts: the first, a slip which is attached to the under surface of the clavicle; the second, consisting of a slip which passes to the inner border of the coracoid process; the third slip having its insertion into the tendon common to the short head of the biceps and the coracobrachialis muscles. In the two muscles which we have noted, this triple form of insertion is clearly suggested, with the exception that on the right side the slip to the clavicle is absent, while on the left side only that to the clavicle is present.



In addition to this the results of observations in our laboratory point to the fact that there is a considerably greater instability of the pectoral musculature in colored subjects than in whites. Also that the greater number of the variations occurring in blacks are of the reversional, while those which have appeared in whites are of the fortuitous, type. This fact, together with the close correspondence of the insertions of the two muscles with those of the gibbon, as described by Hepburn, gives color to the conclusion that we are, in this case, dealing with variations which belong to the reversional type.

Whatever part inheritance may play in the production of variations of the type just described, attention may be called to another factor which is possibly involved in this, and in other variations, which have been observed in relation to the pectoral muscles.

In the development of muscle tendons growth results in an increase in the tensile strength of the tendon fibers and an increase in their length. The first is due to the addition and fixation, in the fiber, of new material; the second involves, in addition to the first factor, an increasing pull on the fibers due to the increase of tonicity in the growing muscle. The direction in which these factors in the growth of tendons operate is from the bone to the muscle.

The reason for the growth of tendons taking place in this direction, when considered from the viewpoint of kinetics, is obvious. In the majority of tendons, mass is greatest near the musculotendinous junction and least near the attachment of the tendon to the bone. An increase in tensile strength of fibers in the area where tendon mass is greatest would cause frequent, if not constant, rupture of the tendon at its weakest point. The fact that the direction of tendon growth is from the bone toward the muscle provides a reinforcement by means of which the danger of rupture at this point, incident on any process of growth, is obviated. The operation of this principle of tendon growth is recognized clinically in tendon transplantation. In all such operations the tendon

is separated as close to the bone as possible for the reason that it has been found to 'grow better' if the separation is made at this point.

In the development of the tendon the increase in the tensile strength of its fibers is accompanied by an increase in length. This is due to the operation of two factors to which reference has already been made: 1) an actual addition of material; 2) the increasing pull on the fibers due to the increase in the tonicity of the muscle. This second factor is one of considerable importance.

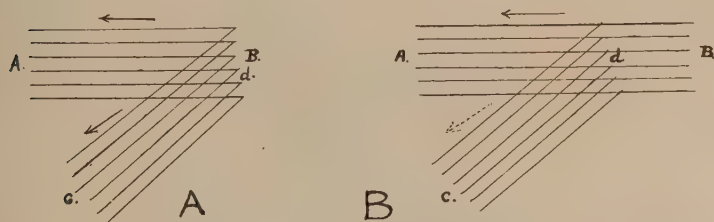


Fig. 1 The lines *AB* and *BC* represent two sets of fibers which occupy the same area of attachment to the bone, *d*. Tendon growth, i.e., increase in length and tensile strength of the fibers, is due to two factors: 1) the addition of new material and, 2) the pull of increasing tonicity in the growing muscle mass. The direction in which these factors operate is from the bone to the muscle. This direction is indicated by arrows; the broken arrow indicating the fibers in which the operation of these factors is least effective. In *A* the operation of these factors in the two sets of muscles is balanced, hence there is no alteration in their relations to the area of attachment. In *B* the factors are operating more effectively in fibers *AB*, hence the fibers *C*, in which growth is relatively slow, are being moved by the more vigorous growth of *AB* to a new point of insertion, *d*.

If these factors, operating in two muscles employing the same area of attachment to a bone, are balanced (normal), the fibers of neither muscle will be disturbed in their original relation to the area of attachment (fig. 1, *A*). If, on the other hand, the operation of these factors is so much greater in respect to the growth of the tendon of one muscle that it cannot be balanced by the operation of similar factors in the growth of the tendon of the other muscle, the fibers of the tendon in which these factors are weak will have a tendency

to migrate from the area of their original attachment, being carried along by the lengthening of the fibers in which the growth factors are operating more effectively. The distance from the area of attachment to the bone which these migrating fibers will travel will depend on the relative difference in the operation of growth in the two tendons (fig. 1, *B*).

While the application of this principle to the extension of the fibers of insertion of the pectoralis minor from the cora-

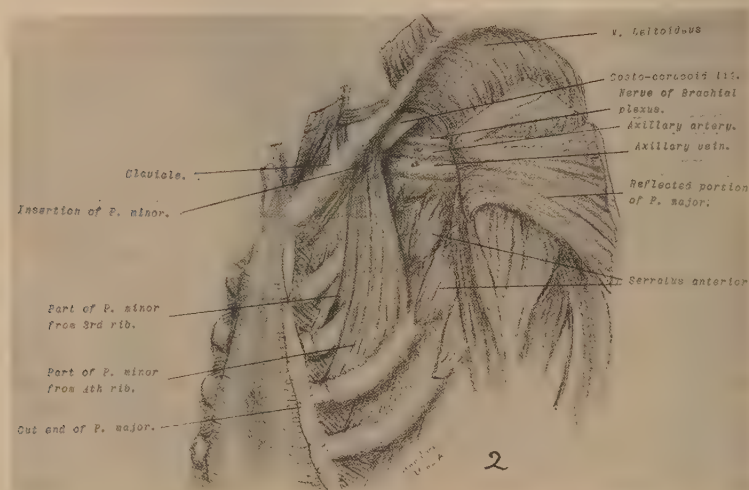


Fig. 2 The drawing was made directly from the subject, the dissection being made so that the form and position of the pectoralis minor on the left side would be clearly shown from its origin from the third and fourth ribs to its insertion into the clavicle and costocoracoid membrane.

coid process to the tendon of the biceps and coracobrachialis muscles, on the right side, seems clear, there might be some question as to its operation in so extensive a migration as is the case with the insertion of the muscle on the left side—a migration so extensive as to change the entire area of insertion of the muscle.

However, the tendinous character of the costocoracoid ligament on the left side would seem to indicate that the factors



of growth in that structure had been operating at an unusual rate; and it is highly probable that in producing the condition observed, the process by which the tendonizing of the fibers of the ligament was brought about proceeded from the coracoid process medialward. The result of this would be that the more slowly developing tendon elements of the pectoralis minor would be borne medially by the lengthening fibers of the membrane, in relation to which they became finally fixed.

It is not to be inferred that this condition represents the only factor in the production of this, or other, variations which have been observed in the muscles of the pectoral region, or elsewhere in the body. For instance, the medial fibers of the pectoralis minor in this case are more or less intermingled with the lateral fibers of the subclavius muscle at the point of their common attachment to the clavicle. This would suggest that an imperfect delamination of the fibers of the pectoralis minor from those of the subclavius, in the development of the muscles of the pectoral group, had operated as an anchor to hold the muscle in this unusual position. The answer to this, however, appears to be that if the factors of tendon growth were operating normally in both directions, the result would be a splitting of the muscle in which a slip, representing the superior portion of the muscle would remain attached to the clavicle in conjunction with the adjacent fibers of the subclavian, while the remainder of the muscle would be directed toward the coracoid process to which its tendon of insertion would be attached. This appears to be precisely what has occurred in the cases reported by Taylor and others.

The extent to which the operation of these factors may be controlled by inheritance, as in the case of reversional variations, presents a problem which we cannot solve by available data. But from the foregoing, and from other observations which I hope to report in the near future, there appears to be sufficient evidence that the operation of the factors of growth in tendons which occupy the same areas of attachment to a bone, when growth is proceeding without

a proper balance in both, plays an important part in the production of some of the variations which appear in relation to the pectoral group of muscles.<sup>1</sup>

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<sup>1</sup>I wish to express my indebtedness to Mr. Ivan C. Berrey for his work in dissecting the muscle and collating the data in reference to it and to Mr. John Sheinin for making the drawing employed in figure 2.

# FURTHER NOTES ON THE ACROSOME OF THE ANIMAL SPERM. THE HOMOLOGIES OF NON-FLAGELLATE SPERMS

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FIVE FIGURES

In several recent papers (Bowen, '22 a, '24 a and b) I have discussed at length the confusing problems connected with the formation of flagellate sperms, and have tried to bring all such sperms under a common viewpoint based on the homologies of the materials from which they are built up. The results of this inquiry (based largely on the work of earlier writers) may be briefly summarized in the following statement of the *rules of sperm formation*.

Broadly speaking (the atypical flagellate sperms exhibit many complications), the flagellate sperm consists of two parts, which may be designated as the head and the tail regions. In the formation of these parts, the original components of the spermatid follow certain definite rules, as follows:

1. The nucleus forms the major portion of the head of the sperm.

2. The Golgi complex gives rise to the acrosome which is always associated in one way or another with the nucleus in the head region of the sperm.

3. The major part of the 'undifferentiated' cytoplasm occupies the interstices between the definitely formed elements in a more or less extensive section of the tail region, sometimes designated as the 'middle-piece.'

4. The centrioles, usually differentiated by position as proximal and distal, assume various configurations in the proxi-



mal (usually) part of the tail region, and the distal (always?) centriole gives rise to the axial filament which forms the structural basis of the entire tail.

5. The mitochondria<sup>1</sup> form a sheath for the tail filament in a larger or smaller portion of its course, this segment being often designated as the 'middle-piece.'

6. The original cell membrane of the spermatid probably remains as the limiting membrane of the sperm.

7. The Golgi complex (with relatively unimportant exceptions), chromatoid bodies (if present), probably remnants of previous spindles, other detritus of sperm formation, and a part of the 'undifferentiated' cytoplasm (the proportion of active cytoplasm here involved is a matter of question) are cast off toward the end of sperm formation and take no structural part in the mature spermatozoon.

Thus, throughout an enormous series of spermatozoa we find that a characteristic flagellate form is developed, which in itself is often sufficient to establish the homologies in some new sperm whose developmental history is unknown.

But in addition to the usual flagellate type of sperm, there is also a much smaller group of sperms which for convenience may be called the non-flagellate type. These non-flagellate sperms form a curious and scattered assemblage which agree in the absence of a tail filament, but disagree in everything else. Indeed, they differ among themselves as much as they differ from the flagellate type. Such sperms are known at present to occur<sup>2</sup> in the Nematoda, Crustacea (particularly

<sup>1</sup>After having spent nearly five years in the continuous study of sperm formation, and having demonstrated the complex transformations of the mitochondrial substance in forming the sheath of the tail filament in a great variety of insect sperms, it is rather disconcerting to find that Gatenby has recently (Gatenby and Bhattacharya, '25) repeated his statement that I have described the atrophy of the mitochondria in the developing sperms of moths, and have traced the origin of the tail sheath to some new substance. It is needless for me to say that no such idea has ever been entertained, let alone described, by me. An explicit statement of the rôle of the mitochondria in the formation of the insect sperm is given in my '22 b paper, q.v.

<sup>2</sup>I have not included the Phalangida, although this should perhaps be done, since, as shown by the researches of Boesenberg ('05), their sperms are more

the Decapoda), chilognathous Myriapoda, and the Acarida. It is interesting to note that these sperms all belong to only two phyla of animals—the round worms and the arthropods. Two general attitudes may be taken toward these curious sperms. On one hand, it might be held that they represent a category of sperms which are to be considered as coequal with the flagellate sperms, with which they may have no very obvious structural relations. On the other hand, most writers have looked upon the non-flagellate sperms as being essentially flagellate sperms which have merely undergone a process of foreshortening without the destruction of homologies with various regional subdivisions of the flagellate type.

The first of these views, in so far as it might tend to separate the two types of sperms too sharply, is of course theoretically unsound, since non-flagellate sperms occur in many animals whose immediate relatives have typical flagellate sperms. Furthermore, the sperms of sponges and coelenterates, not to mention the Protozoa and lower plants, are of the flagellate type, and everything points to the conclusion that this alone was the primitive condition. It seems, therefore, that non-flagellate sperms have appeared sporadically in animals whose ancestors formed sperms of the usual flagellate type. There can thus be no phylogenetic relationship between the various kinds of non-flagellate sperms, and the conclusion seems inescapable that they have descended from the flagellate type of sperm, which for quite inexplicable reasons was remodeled into the various bizarre kinds of non-flagellate sperms. In so far, however, as this first point of view expresses merely the fundamental differences in structural relationships and seeks to isolate the non-flagellate sperms as a matter of convenience, it would seem to have much more in its favor than has generally been admitted.

Nevertheless, it does not follow from this that the second theory is necessarily correct. Indeed, to cite the simple case

nearly non-flagellate than those of some of the Diplopoda. This is a matter of little consequence here, since so little is known of their make-up that they cannot as yet be profitably discussed.

of the sperms of *Oxyuris* and *Ascaris* (fig. 2B and C) (to be considered in detail later on), endless confusion has been caused by the effort to trace their relation to flagellate sperms in which the tail region has been much shortened. To put the matter concisely, workers who have accepted this alternative explanation have directed their efforts toward establishing the homologies of the head and tail regions *as such* in the non-flagellate sperms, with much too little attention to the disposition of the various components of the spermatid, considered quite apart from their mere location in the flagellate sperm as described in the terms head, tail, etc. It need scarcely be said that while the derivation of the non-flagellate from the flagellate sperms must in some sense be accepted as theoretically correct, it does not follow at all that an understanding of the non-flagellate sperms must or can depend on comparisons of their parts with the head and tail regions of the flagellate type. In other words, while theoretically correct, as a manner of viewing the non-flagellate sperm, this explanation has in actual practice failed to yield any criterion for understanding them. The truth seems to be that these sperms have descended from flagellate types, but that this past relation is of little use per se in understanding their present morphology, which must be approached each time from the standpoint of the peculiarities of the particular sperm, and not from that of the flagellate arrangement of parts.

I propose, therefore, to discard much of the substance of both the old ways of viewing the non-flagellate sperms and to approach the whole problem of their meaning from a somewhat different standpoint. It is my belief that the known groups of non-flagellate sperms have descended separately and quite independently from ancestral flagellated types. They have, accordingly, no reason for being classed together other than that of convenience in description. But, further, I believe that the solution of the whole problem must be sought not in the view that the non-flagellate sperms are essentially foreshortened sperms of the flagellate type or, on

the other hand, that they are necessarily something quite different from flagellate sperms, but rather in the general view that the sperm merely represents a way of putting certain essential components into a compact and convenient packet. In the majority of cases this has led to the development of a flagellate form, but in a few cases this form has been more or less completely discarded and the component parts have been put together in ways which need not necessarily be like, or even regionally related to, those characteristic of the flagellate sperm. Even among the atypical flagellate sperms, this possibility of the rearrangement of materials as such, rather than a mere transposition of regional parts, is already evident, as illustrated by the absurd position of the acrosome in *Lepisma* (fig. 2D). In other words, my opinion is that homologies between sperms are not to be established between head, neck, middle-piece, tail, etc., but between the materials, the morphological components, of these parts. The head of a flagellate sperm does not make the nucleus—the nucleus makes the head; the tail does not make the mitochondria—the mitochondria make the tail sheath, etc.

On this basis I propose to review the non-flagellate sperm types, in the hope of clearing up something of the confusion which now invests the whole subject. Whether my conclusions be correct can be decided only by further observation, but I hope at least to show that the method of attack here suggested is the only one which promises eventual success. From what I have said, it will be unnecessary to draw strained comparisons between the regions of different sperm types, but merely to make out the disposition in the non-flagellate sperms of the components whose arrangement in the flagellate sperm has been noted in the earlier statement of the rules of sperm formation. For, in my view, the possession of these various components is the one really basic similarity shared by all sperms.

The non-flagellate sperms which have been most extensively studied are those of the nematodes, and the discussion will accordingly begin with them.



## NEMATODA

Of the thousands of species of nematodes, only a few have been examined from the standpoint either of sperm formation or the morphology of the mature sperm. Those which have been studied exhibit a general structural similarity, and all agree especially in the simplicity of their construction. They are indeed by all means the simplest of the non-flagellate sperms. Incomplete accounts of sperm formation have been given for *Strongylus* by Struckmann ('06) and for *Sclerosotomum* by Kuehtz ('13), while a much more extended description for *Oxyuris ambigua* has been worked out by Loewenthal ('89, '90) and Meves ('20). Fragmentary information concerning the sperm of *Filaria* (Meves, '15) and other nematodes has also been accumulated. But the most extensively studied of the nematode sperms, indeed of sperms of any kind, is that of *Ascaris*.<sup>3</sup> Beginning with Van Beneden and Julin ('84), a long series of workers have attacked the problem. A brief paper by Hirschler ('13) is in most respects the best of them all. Considering the structural simplicity of the *Ascaris* sperm, the differences in the various accounts are rather astonishing, and most of them will be dismissed without mention—provided the correct alternative is reasonably certain. The interested reader can find a complete bibliography in Hirschler's paper and a very extensive comparative critique in the review of Romieu ('11).

In spite of several findings to the contrary, it now seems certain that the *Ascaris* sperm normally completes its development in the male sexual ducts, and not in those of the female, as was contended by Van Beneden and Julin. It is also clear that of the four sperm types described by the same authors, only one, the 'type conoide,' is to be looked upon as a normal, functional sperm.

All things considered, the *Ascaris* sperm seems to be made up as follows: The general shape is that of a blunt cone (fig.

<sup>3</sup> Several different species of *Ascaris* have been studied, but in all cases the sperms seem to be essentially identical. No effort will be made, therefore, to distinguish species in this discussion.

2C), the sperm penetrating the egg with the blunt end, which is therefore to be considered as the anterior or 'head' end. The basis of this cone is formed by a part of the undifferentiated cytoplasm of the spermatid, the remainder being cast out, as is usual in sperm formation (see particularly Hirschler, '13). The nucleus is represented by a small, structureless sphere in the anterior region of the sperm, more or less enclosed by the mitochondria which occur in the form of scattered granules, some of them being located in the more posterior cytoplasmic parts as well. The location of the centrioles is still a matter of some uncertainty. In the figure I have provisionally placed them inside the nucleus, following somewhat inconclusive accounts by several workers. This location has undoubtedly been favored by several descriptions which assign to the centrioles of the maturation division figures an intranuclear source. By others, this origin has been categorically denied. The whole question is in a decidedly unsettled state, and for my present purposes it is not essential to reach any final decision now. A real perforatorium or acrosome has been found only by Scheben ('05), who described the production of a finely modeled acrosome by a 'sphere,' in more or less accordance with the customary procedure. This acrosome was located at the pointed end of the cone. It has never been seen by any one else, but Mayer ('08) found a body in the same general location which he described as a chromatoid body, and Romieu ('11) considered a somewhat similar structure as a most extraordinary nuclear derivative—a rather special centrosomal formation in which he found the homologue of the intermediate piece of typical sperms. Hirschler's ('13) observations show that this body is really the remnant (probably poorly fixed in the preparations of other workers) of the Golgi apparatus left behind, as in the case of the mammalian sperm, when the residue of the Golgi complex is cast off (fig. 1D and E and fig. 2A and C).

Thus far the components of the *Ascaris* sperm present nothing new or unusual. But there remains one other struc-

ture, the largest one in fact of the entire sperm—the so-called refringent body. This has been known as the corps réfringent, Glanzkoerper, Fettkoerper, Kopfkappe, Schwanzkappe, etc., according to the theoretical prepossessions of the author. It is a large (hollow?) conical mass, whose outstanding peculiarity is its glistening appearance when seen in the living sperm. Concerning the origin and function of this material there has been endless speculation, one modern author, Scheben ('05) even finding in it the achromatic structures of the nucleus. To cut short a very long story, it may be said that up to the present time no one has made even a satisfactory guess as to the meaning of the refringent body. It is my belief, and it is largely the purpose of this paper to develop an argument in favor of the view, that the material of the refringent body is nothing more or less than the substance out of which in flagellate sperms the so-called acrosome<sup>4</sup> is constructed. If at first blush this conclusion seem absurd, I can only ask the reader to follow the considerations which in my opinion admit of no other interpretation.

In a recent series of papers on the sperm formation of insects and amphibia, I have stated and developed the thesis that the material constituting the so-called acrosome in flagellate sperms is invariably a differentiation product of the Golgi complex. Indeed, approaching the problem from the opposite direction, we might even say that to flagellate sperms generally the Golgi complex always contributes a specific substance which happens to form what has come to be known as the acrosome. In other words, it is another case of the Golgi product forming the acrosome, and not the other way round. Against this generalization no serious objection or exception has yet been raised, so far as I am aware. It follows, therefore, that in all sperms there should be some component equivalent in respect to origin to the acrosome of the

<sup>4</sup>It is interesting to note that Nussbaum ('84) long ago called the refringent body the 'Kopfkappe,' a terminology which seemed illogical to Zacharias ('87) because this material occupies the 'tail portion' of the sperm. It goes without saying that neither writer had any notion of the modern implications of the acrosomal part of the sperm.

flagellate sperm, but not necessarily occupying the position of a perforatorium. In the *Ascaris* sperm the refringent body is the only component not otherwise traceable to one or another constituent of the spermatid, and the conclusion would seem to follow that this body must therefore be the customary derivative of the Golgi complex.

Following up this argument by exclusion, to which no finality can of course be attached, my contention should find support, if it be true, in the origin of the refringent material and in its general behavior during spermatogenesis. It is generally agreed that the material of the refringent body makes its first appearance in the cytoplasm of the primary spermatocytes during the early growth period, in the form of granules. These granules occur widely scattered throughout the cytoplasm. They are much larger than the mitochondrial granules with which they cannot possibly be confused (fig. 1A), and are easily recognized in the living sperm by their shining appearance. Their number becomes eventually very large, and they crowd the whole cytoplasmic area (fig. 1B). At the time of the spermatocyte divisions their shape becomes ovoid, and the larger ones divide to form smaller ones. Dozens of these bodies, rather uniform in size, are thus produced. Meanwhile the mitochondrial granules, by processes of fusion and elongation, give rise to numbers of small rodlets which become applied to the ovoid refringent granules, a single rod being associated with each granule (fig. 1B). The rods and granules now become oriented around the centrioles of the division figure in a most extraordinary manner, and are thus distributed with approximate equality to the daughter cells. During interkinesis the rods and granules divide, so that the ensembles are markedly smaller in the second division than they were in the first. In the spermatid, the relation between the mitochondria and the refringent granules is again dissolved, the mitochondria returning to their original form of scattered granules (fig. 1C to E). The refringent granules, on the other hand, begin to fuse together, and this eventually results in the formation



of a single mass, which is modeled into the conical refringent body of the mature sperm (fig. 1C to E and fig. 2C). This remarkable history was first made out in any detail by Tretjakoff ('05), whose findings have been corroborated by other workers, particularly Hirschler ('13), who extended them in several important directions.

Retracing our steps, it is necessary to inquire now into the details of the nature of this material and its origin. Due to its glistening appearance, the conclusion has been frequently drawn that the refringent material is a fatty substance. Thus Marcus ('06) considers it as yolk which serves as a nutritive material for the sperm. A similar view of its function has been held by others. Romieu ('11) considered the material of the refringent body to be a nucleo-albumen, and its function that of a protection for the nucleus and a fixed point in the amoeboid movements supposed by him to be attendant upon the efforts of the sperm to penetrate the egg. It is useless, however, to continue a review of these speculations. The function of the refringent body is still in no way agreed upon, and its chemical nature is unknown, except that it is clearly not a simple lipoid material to be compared with an inert mass of yolk. In any event, nothing which throws any light on the problem as a whole is known about this material, or for that matter about the material which makes up the acrosome of flagellate sperms.

With respect to the origin of the refringent granules, nothing very definite has yet been made out. They are usually referred to an indefinite cytoplasmic origin, and one author, Wildman ('13), traced them to an ultimate nuclear origin. Hirschler ('13) alone has presented any serious analysis of this question. He believes that the refringent granules arise by a process of direct transformation from the chondriosomes. His evidence is quite inconclusive, however, and seems to depend largely on the histogenetic possibilities of mitochondria so extensively exploited in recent years by Meves and many others. I think it fair to conclude that the origin of the refringent granules must still be considered as unknown.

If the homology which I have suggested between the refringent substance and the acrosomal material of flagellate sperms be correct, it should be possible to trace the origin of the refringent granules to the Golgi apparatus. This raises at once the whole question of the form and behavior of the

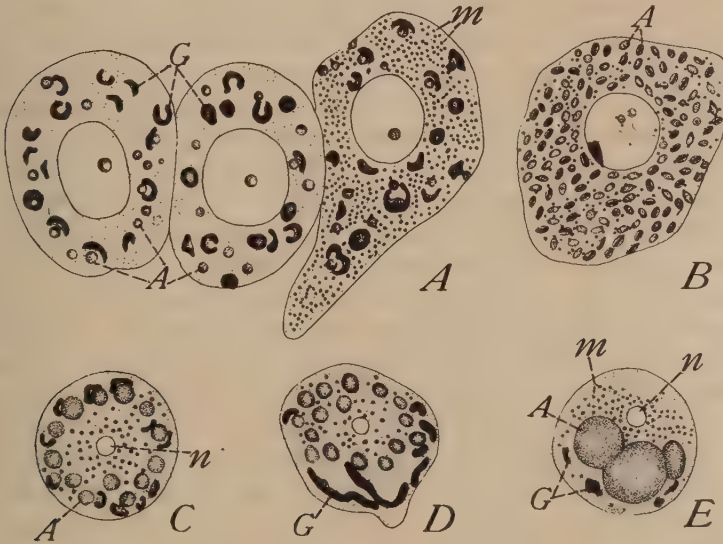


Fig. 1 Stages in the spermatogenesis of *Ascaris* sp. A, spermatocytes in the growth period, showing arrangement of Golgi bodies and refringent granules, with mitochondria also shown in right-hand cell (after Hirschler); B, spermatocyte preparing for the first maturation division (slightly schematized after Tretjakoff); C to E, spermatids showing steps in fusion of the refringent granules to form the refringent body, with simultaneous fusion and elimination of most of the Golgi apparatus (after Hirschler). A, refringent granules or material (= acrosome?); G, Golgi material; m, mitochondria; n, nucleus.

Golgi material in the *Ascaris* sperm cells. Fortunately, this history has been rather completely worked out by Hirschler himself in *Ascaris lumbricoides*. The Golgi apparatus occurs in the spermatogonia in the form of scattered pieces very similar in appearance to the scattered Golgi bodies of insects. They are distributed to the daughter cells of the spermato-

gonial divisions in approximately equal amount, and thus finally arrive in the primary spermatocytes still in the form of discrete Golgi bodies. During the growth period these increase in number and size and give to the spermatocytes an appearance strikingly similar to that of an hemipteran spermatocyte of the same stage (fig. 1A). They are distributed to the spermatids, by way of the maturation divisions, in approximately equal quantities. In the spermatid (fig. 1C) they presently begin a process of fusion, running together to form long, bulky rods, with usually a few isolated remnants of small size (fig. 1D). When the protoplasmic balls are cast off, as happens so generally in flagellate sperms, the great bulk of the Golgi material goes with it. There are left behind in the sperm only a few remnants (fig. 1E), which eventually occupy a place near the point of the conical sperm (fig. 2C) and are obviously homologous with the remnant of the Golgi apparatus left behind in the mammalian sperm, as first made out by Perroncito ('10), Weigl ('12), and Kolmer ('16). In its general behavior, therefore, the Golgi apparatus follows the usual history in sperm cells.

Hirschler apparently did not consider the possibility of any relation between the Golgi bodies and the refringent substance. But it occurred to me that if my conception of this substance was correct, it ought to be possible to find some cogent evidence in Hirschler's figures. Upon examination, I was astonished to find that in his figure 4 of the spermatocyte growth period, there was a really remarkable number of cases in which single refringent granules were clearly associated with single Golgi bodies (fig. 1A). The Golgi body more or less embraces the refringent granule in most cases, and the ensemble seems altogether too close a replica of the actual conditions attending the differentiation of the acrosome in the flagellate sperm to be dismissed as a mere coincidence. It is all the more astonishing that Hirschler's figures should show this relation so clearly when he himself had in mind no theoretical bias in favor of such an interpretation. I am inclined, therefore, to draw the following con-

clusion, which must, of course, be subject to revision if further observations make it untenable. The refringent granules arise as differentiation products of the Golgi apparatus in a manner essentially comparable to that by which normally the acrosome in the spermatid takes its origin. As the granules are formed their relation to the Golgi bodies is dissolved and they collect in large numbers in the cytoplasm. Their size at the time of leaving the Golgi bodies very likely varies, and it is quite possible that the larger refringent granules may sometimes be fusion products of smaller granules already separated from the Golgi material. Their peculiar relation to the mitochondria at the time of the maturation divisions is probably of no general significance, and is to be looked upon merely as a peculiarity interesting only as it bears on the larger problem of cytoplasmic distribution during mitosis. Arrived in the spermatid, there is again a frequent close relation between the fusing masses of refringent substance and the Golgi bodies (fig. 1C), but this relation at best is very brief and probably plays no important rôle in the further elaboration of the refringent substance.

It remains now to inquire whether the history here outlined for the refringent, or as we may better call them, the proacrosomal granules has any certain counterpart in the spermatogenesis of typical flagellate sperms. If such a counterpart could be established in flagellate sperm formation, it would obviously be exceedingly difficult to raise any serious objection to the interpretation here proposed for the condition in *Ascaris*. It is, therefore, of extraordinary interest that a parallel case has long been known in mammalian spermatogenesis, the complete details of which have been recently worked out by Papanicolaou and Stockard ('18) in the Guinea-pig. Here the Golgi apparatus is concentrated at one pole of the cell associated with the customary idiosomic material. Within the idiosome many small granules are developed which at the time of the maturation divisions are released into the cytoplasm. During interkinesis and finally in the spermatid the idiosome is reconstituted and the ('proacro-



somal') granules return to their position within it. In the spermatid stage the granules increase rapidly in size and gradually merge to form the acrosome which is finally deposited on the sperm head, while the Golgi remnant (acroblast = Golgi material plus idiosomic substance) is cast off. This history is identical with that of the refringent granules in *Ascaris*, except that the size of the acrosome being relatively smaller, and the Golgi complex being concentrated instead of scattered, there results a less conspicuous development of the proacrosomal granules during the spermatocyte stage. Furthermore, that the separate pieces of the Golgi complex can independently produce acrosomal material and in a quantity proportional to the size of the Golgi piece is indicated by the conditions which probably occur in the *Lepidoptera* (Bowen, '22 c), where the Golgi bodies are always scattered (multiple acroblast, Bowen, '22 a) and separately contribute their quotas to the acrosomal mass. Here, too, attention may be called to the occasional abnormal conditions found in forms where the Golgi complex is usually single (Bowen, '22 b, fig. 54). In this chain of evidence there is only lacking a case in which the Golgi apparatus in the spermatocytes is scattered and the proacrosomal granules correspondingly distributed throughout the cell as in *Ascaris*.<sup>5</sup> But I will venture the prediction that in cases where the acrosome is unusually large, particularly as compared to the amount of Golgi material, the formation of proacrosomal granules in the primary spermatocyte will be found to be a normal and customary procedure. Thus what has seemed a peculiarity of mammalian sperm formation appears now to be in reality a fairly widespread phenomenon which represents only another complication of a process which remains fundamentally the same. The more the way of forming the acrosome changes, the more it remains the same thing.

I therefore conclude that the refringent body in the *Ascaris* sperm is the homologue of the so-called acrosome in the flagel-

<sup>5</sup> That such cases occur (and probably with some frequency) is indicated by some unpublished work with which I am acquainted, in which exactly this state of affairs seems to obtain.

late sperm not by virtue of its position in the sperm, but because it seems to follow the established rule for the formation of acrosomal material. The only difference is, that in the *Ascaris* sperm the term acrosome is more of a misnomer than usual.

Before coming to the conclusion of this section, I will mention briefly the sperm of another nematode, *Oxyuris ambigua*, whose formation was long ago studied by Loewenthal ('89) and more recently by Meves ('20). The general arrangement of parts is indicated in figure 2*B*. The mitochondria form a long, attenuated appendage which is physiologically posterior. In front is attached a large protoplasmic ball containing a small nucleus and probably the centrioles, though the exact arrangement of the latter is uncertain. No refringent body has been clearly described in this sperm, but I suspect that it is actually present in the large protoplasmic head-piece, which is otherwise inexplicable. Furthermore, Loewenthal figures ('89, fig. 27*b*) a developing sperm with the cytoplasmic mass loaded with vesicles bearing every resemblance to the refringent masses in *Ascaris*, and later he describes the 'finely-granular contents' of the anterior protoplasmic mass as something separate from the enclosing undifferentiated cytoplasm. This is indicated in the diagram (fig. 2*B*), and there is every reason to believe that this mass represents the refringent material, which in this case is much less conspicuous throughout the cycle than it is in *Ascaris*.

I come now finally to the consideration of the old question of the homologies of sperm parts with the head and tail regions of the flagellate type. It happens that the sperm of *Oxyuris* has long been used as an example of the relation between the elongate flagellate sperm and its supposedly foreshortened, non-flagellate relatives. As a matter of fact, the sperm of *Oxyuris* does fit in to such a scheme in a remarkable way, for it is known that the mitochondrial appendage actually is the posterior part of the sperm. Its obvious relations to the schematized flagellate condition are brought

out in figure 2*A* and *B*. But when we extend this comparison to the sperm of *Ascaris*, the whole conception is seriously compromised, for here the so-called tail section does not con-

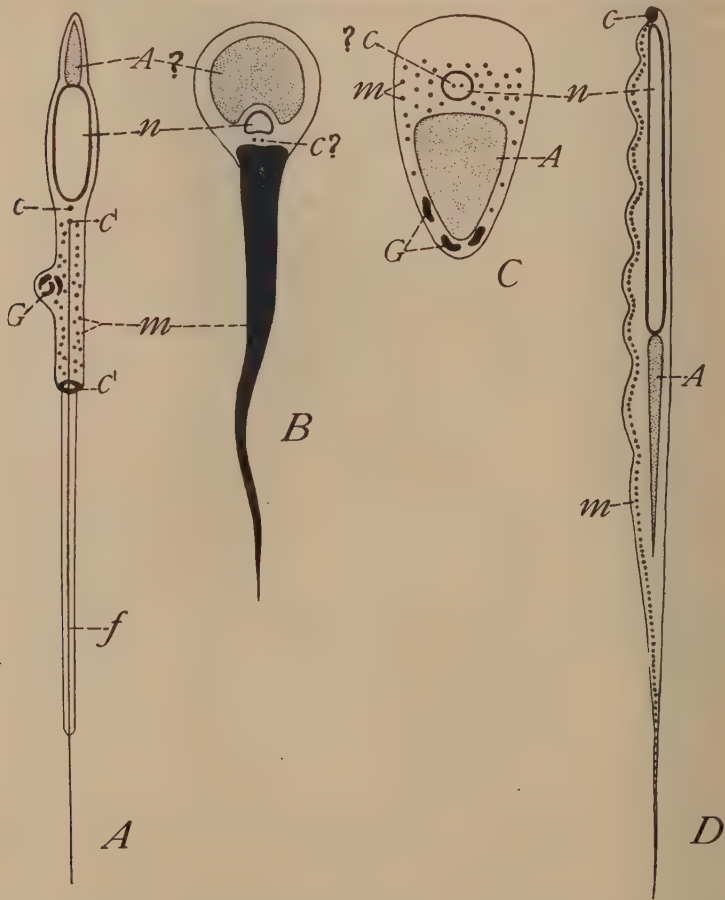


Fig. 2 Diagrammatic comparison of nematode sperms with flagellated types; the sperms are arranged with the physiologically anterior ends at the top (the condition in *Lepisma*, however, is not positively known). *A*, schematized flagellate sperm, based largely on mammalian conditions; *B*, sperm of *Oxyuris*; *C*, sperm of *Ascaris*; *D*, sperm of *Lepisma* (atypical flagellate type). *c*, centrioles (in *A*, *c* is the proximal centriole; *c'*, the derivatives of the distal centriole); *f*, tail filament; other lettering as in preceding figure.

tain the mitochondria, but the refringent body (=acrosome?). Even here, however, the situation can be apparently saved by a comparison with the sperm of *Lepisma* (Bowen, '24 a) in which the acrosome is likewise curiously reversed in respect to the sperm nucleus (=head) (fig. 2C and D). But to my mind, such comparisons must always appear very strained, indeed, and these superficial resemblances to flagellate sperms are at best deceptive. In fact, the attempt to explain the nematode sperm on the basis of comparison with the head and tail regions of flagellate sperms has led only to confusion and a complete misconception of the real state of affairs.

It seems to me, therefore, as I stated in the introductory pages of this paper that, so far as the non-flagellate sperms are concerned, we had best discard the dangerous application of the convenient descriptive phrases used in connection with flagellate sperms. I look upon sperms merely as varied solutions of the problem of stowing away a variety of definite substances in a compact form, and while this form was once of a flagellate type, it does not follow that comparison with the ancestral form will give any valuable clue to the solution of the form of its descendants. I can only reiterate, in a still broader sense, the conclusion to which I came in a former paper (Bowen, '24 a), "The . . . sperm seems never to transgress the few rules which govern the production of its fundamental parts, but in the arrangement of these parts every sperm" (flagellate or non-flagellate) "seems to be a law unto itself."

#### CRUSTACEA—DECAPODA

Several groups of Crustacea are characterized by non-flagellate sperms, but the only forms which have been very thoroughly investigated are the decapods, and this section will deal only with them. Among the decapods there are two chief types of spermatozoa. One, characteristic of the *Macrura* and some of the *Anomura*, is a somewhat elongate structure, from the middle of which a series of long, stiff processes



(the typical number is three) extends (fig. 3*A*). The other, characteristic of the *Brachyura*, has a spherical central part from which irregular processes radiate (fig. 3*B*). The formation of these sperms has been extensively studied by Koltzoff ('06), whose account, unfortunately, commences only with the maturation divisions.

According to Koltzoff, who seems to have been dominated by the necessity of homologizing head, neck, and tail regions with those of the flagellate sperm, the physiologically anterior part of the sperm represents the head (= nucleus); the posterior portion—the so-called capsule—represents the tail, while the intermediate connecting region is the homologue of the neck. The centrioles occupy a characteristic position within the capsule as indicated in the diagram (fig. 3). The mitochondria are present both in the head and neck regions, and although they differ somewhat in the technical details of their demonstration, Koltzoff believes that they are really one and the same thing. They form a 'skeletal' support for the head and the characteristic processes. In one case (fig. 3*A*) the processes spring from the neck, in the other (fig. 3*B*), from the head. According to Koltzoff there is no perforatorium, for the reason that at no stage in the development of the sperm are there any traces of a centrotheca (idiosome). These statements together with the diagrams in figure 3 will suffice to give a general idea of these sperms.

The question of interest here centers around the nature of the so-called capsule and its contents—a structure apparently without a homologue in any other kind of sperm.

Examining this structure more closely, we find that it owes its origin to the gradual fusion of a large number of granules or droplets, which appear in the primary spermatocyte and are distributed, during the maturation divisions, to the spermatids. These droplets are readily visible in the living cell, and are easily distinguishable from the mitochondria. Sometimes they are astonishingly numerous and are crowded into every available space in the spermatid. Koltzoff called them capsule or tail granules. This brief description will

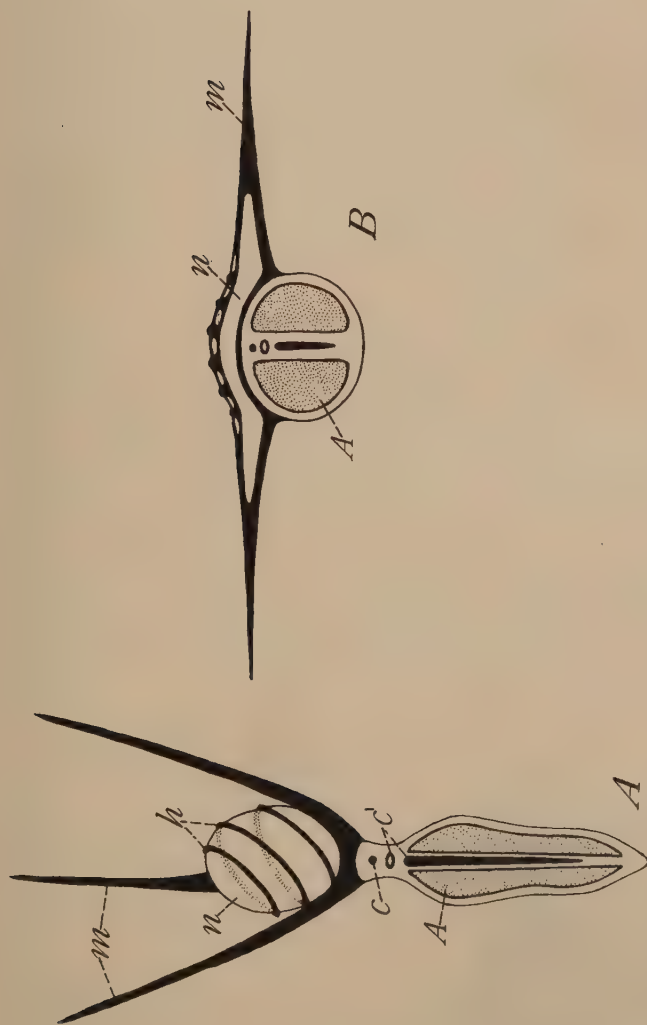


Fig. 3 Diagrammatic illustrations of decapod sperms (based largely on Koltzoff). *A*, macruran type; *B*, brachyuran type. *A*, 'capsule' (= refringent material or acrosome<sup>†</sup>); *h*, skeletal fiber of nuclear region, presumably of mitochondrial origin; *c*, proximal centriole; *c'*, derivatives of distal centriole (arrangement of centrioles in the two types is exactly the same); other lettering as in preceding figures.

hardly fail to suggest at once the probable nature of these granules. They are, in my opinion, homologous with the acrosomal material in other sperms. As a matter of fact, more than one worker on the nematode sperm has considered these granules in the decapod sperm cells as directly homologous with the refringent granules of *Ascaris*. There is thus a considerable background of similarities which, if they are lacking in conclusiveness, nevertheless offer a real basis of comparison. Unfortunately, nothing is known concerning the Golgi apparatus in these decapod sperm cells, and it is therefore impossible at present to do more than urge the comparisons already made.

To summarize the conditions in the decapod sperms, it would seem that the acrosomal material is represented by the so-called tail or capsule granules, which in this case undergo a very unusual differentiation, resulting in the formation of the 'capsule' of the mature sperm. This unusual behavior need arouse no more comment than the equally aberrant arrangement of the mitochondrial components. Further, it seems probable that the efforts, particularly emphasized by Koltzoff, to homologize the parts of the decapod sperm with those of a flagellate sperm are futile, and that the better way is to view these sperms as merely another way of putting together a few fundamental constituents.

#### MYRIAPODA--DIPLOPODA

The study of these sperms by modern methods has as yet been very limited, and the basis for possible comparisons is correspondingly insecure. I wish merely to indicate very briefly what seems to me a possible explanation for these very curious sperms. So far as I know, only two genera have been carefully examined, *Pachyiulus* by Oettinger ('09), and *Polyxenus* by Sokolow ('18). Curiously enough, even between these two genera there are extraordinary superficial differences. The sperm of *Pachyiulus* is more or less hemispherical or bluntly conical in shape, being usually likened to a helmet, from the peak of which extends a long flagellum

(fig. 4*B*). Strictly speaking, this might be sufficient ground for classifying it as a flagellate sperm, but in every other feature it is clearly related to non-flagellate types. It belongs, indeed, in much the same class as the sperms of the Phalangida, which possess a 'tail' filament, but in other respects would seem best considered with the typical forms of non-flagellate sperm. The spermatid of *Polyxenus* undergoes a most remarkable and complicated development, appearing when mature in the efferent male ducts as a sausage-shaped body—or, as Sokolow terms it, a 'diatomiform' spermatozoon (fig. 4*A*). But in the seminal receptacle of the female these sperms undergo a sort of 'conclusive ripening,' in which each is transformed into a long coiled spiral, which, when unwound, would have a somewhat ribbon-like shape. These final stages have been very incompletely analyzed, and I have therefore taken the diatomiform stage as a basis for comparison, and this is shown, in cross-section, in figure 4*A*. In figure 4 both sperms have cast off the protoplasmic balls which were originally attached along the entire ventral surface of each sperm.

There are certain obvious comparisons such as the median location of the nucleus in which chromatic areas are very definitely localized in characteristic patterns. Between the nucleus and the upper surface, the centriolar apparatus is disposed, on both sides of which and extending down over the nuclear wall the mitochondria are massed. Along the ventral face of the sperm of *Pachyiulus* an extensive deposition of cytoplasmic granules occurs, which in my figure are arbitrarily represented as they appear in an immature condition. In *Polyxenus* a somewhat similar appearance is produced by the so-called 'valves' which run along both lateral edges of the sperm and have the same honeycomb appearance which is less clearly exhibited in *Pachyiulus*. Both by a process of elimination and by their general appearance and history in the sperm cells, I conclude that, whatever these substances may be, they are to be looked upon as essentially homologous—a view which was tentatively sponsored also by Sokolow.



According to Oettinger, the mitochondria in *Pachyiulus* are of two kinds—the centrosomal mitochondria which accumulate around the centrioles (*m* in fig. 4*B*) and the thread-form mitochondria which are segregated independently in the spermatid and give rise to the ventrally placed (honeycomb) substance just mentioned (*A* in fig. 4*B*). The centrosomal mitochondria are clearly equivalent to the usual mitochondria of sperm cells. The other type are traced to a material which Oettinger says is mitochondrial, but his figures indicate in the clearest manner that it is really the Golgi apparatus (his figs. 43 to 47). This material was not clearly followed through the intermediate stages of sperm formation, but from it eventually arises a great number of small granules or droplets which are exhibited with great clearness in living spermatids. These are developed in the ventral protoplasmic mass which is later lost, and from this location they stream upward to the ventral surface of the developing sperm proper and here form the honeycomb plate. They leave behind some degenerate remnants (Oettinger's fig. 84), which are later lost together with the protoplasmic mass.

Omitting for the moment any comment on these results, we find that Sokolow found likewise in *Polyxenus* two kinds of mitochondria. One type was finely granular and early became arranged along the developing centriole—an obvious homologue of Oettinger's 'centrosomal' mitochondria (*m* in fig. 4*A*). The other type, which Sokolow apparently does not differentiate until the spermatid stage, is ranged around the entire periphery of the spermatid in extraordinary numbers. At first each has the appearance of a deeply stained granule, which soon increases in size and inside of each one appears an eccentric cavity containing a granule.<sup>6</sup> Later these cavities become larger, stain less strongly, and each body gradually elongating, the ensemble gives rise to an alveolar appearance comparable to the honeycomb in *Pachyiulus*. It is these elongate bodies which form the 'valves' of the sperm (*A* in fig. 4*A*). In addition, there is in these sperm cells an abun-

<sup>6</sup> Note here the amazing similarity to the differentiation of the vesicular type of acrosome in typical flagellate sperms.

dant Golgi apparatus (Sokolow's spherosomes), which has a rather typical history (except that he derives it from mitochondria), and is eventually cast out with the protoplasmic balls.

How is this 'honeycomb' substance to be interpreted? In the first place, the description of two kinds of mitochondria totally different in their behavior is a complete anomaly in

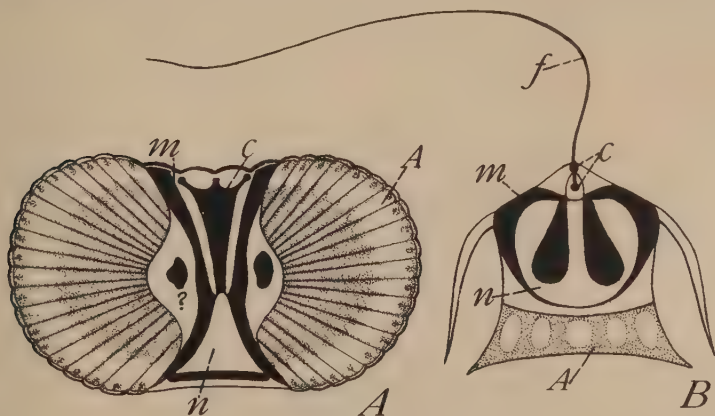


Fig. 4 Sperms of chilograthous myriapods. *A*, cross-section of diatomiform sperm of *Polyxenus* (slightly schematized after Sokolow); *B*, sperm of *Pachyiulus* (schematized after Oettinger). *A*, material (= acrosome?) making up the 'honeycomb' plate and valves (in *A* the individual tubes (cut in longitudinal section) derived from the spermatid granules, each present the characteristic differentiation of the typical acrosomal vesicle); *n*, nucleus, containing peculiar chromatic bodies in *Pachyiulus*, and with a chromatic basal plate in *Polyxenus*; (?) refers to a region in the sperm of *Polyxenus*, the significance of which is unknown; other lettering as in preceding figures.

sperm formation, and in my opinion this fact alone is enough to indicate that the true relations of this material have been overlooked. But if we try to analyze the accounts of Oettinger and Sokolow we at once meet the familiar description of the visibility and high refractive properties of these granules in the spermatid. Further, in *Pachyiulus* the substance which gives rise to the granules, although obviously poorly fixed, has every appearance in the early spermatids of being

the Golgi complex. The origin of the granules in association with this material and the leaving behind of a remnant to be cast off with the protoplasmic ball, all point to only one conclusion. The material which forms the ventral alveolar plate in the *Pachyiulus* sperm is the homologue of the acrosomal material in flagellate sperms. And it is interesting to note that Oettinger himself considered the possibility of its relation to the molluscan Golgi apparatus (then called the 'Nebenkern'), but dismissed the matter, because its position in the spermatid was impossible for the 'sphere'—again the old trouble of looking always for the homologies of position instead of substance, between flagellate and non-flagellate sperms. In *Polyxenus* the facts are more obscure, but the enormous number of granules which go to make up the valves suggests that possibly they were being formed during the spermatocyte stages as in *Ascaris*, but were overlooked (Sokolow, pl. II, figs. 30, 35, and 40). On the other hand, the quantity of Golgi apparatus in the spermatids is extraordinarily great, and again the relation of the granules to it may have been missed, although they were actually being produced in the usual manner. That the real origin of these granules might readily have been overlooked is indicated by the fact that Sokolow never represents them in a clearly stained and distinguishable form until they are arranged on the cell periphery, and even then in only a few cases. I suspect that their existence in earlier stages was thus unrecognized because of technical insufficiencies. I believe, furthermore, that their many points of similarity with probable acrosomal material in other highly bizarre relations justifies their tentative acceptance as the homologue of the acrosomal material of flagellate sperms.

It thus appears that these sperms, while hopelessly lacking in homologies with the segments of the flagellate sperm, nevertheless exhibit again all the characteristic homologies between the original components of the spermatid. I confidently believe that further and more critical studies of the myriapod sperms will establish the validity of this tentative conclusion.

## ARACHNIDA—ACARIDA

The spermatozoa of the Acarida have in many respects the most extraordinary developmental history known. It has recently been studied again in *Argas* by Casteel ('17), whose results are in many respects very complete, but are lacking entirely as regards the centrioles (a matter not important for my purposes) and the details of the Golgi apparatus, in the spermatid. I will, therefore, merely call attention to conditions in the early spermatid without attempting to analyze the mature sperms.

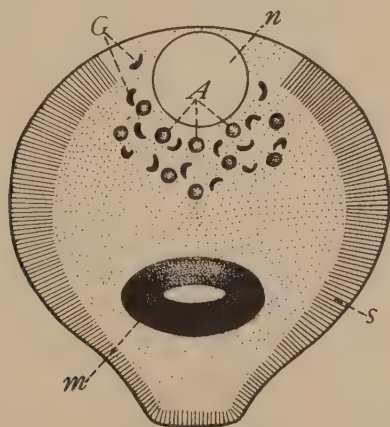


Fig. 5 Spermatid of the acarid, *Argas* (slightly schematized after Casteel). *s*, striated border; other lettering as in preceding figures.

A young spermatid is shown in figure 5. The nucleus is very eccentrically placed, and at the opposite side of the cell is located a large, heavy ring of granular mitochondria. Between them, but more closely associated with the nucleus, is a number of scattered, darkly stained pieces which Casteel terms the 'vesicular bodies.' These he relates to mitochondria, but his figures indicate their undoubted status as the Golgi apparatus in the scattered condition so typical of arthropods. In the early spermatid (fig. 5) there appears among these Golgi bodies a number of clear, transparent droplets



which Casteel calls 'oil droplets.' For this term there is no apparent basis except that of appearance. He suggests that these droplets may have arisen from the 'vesicular bodies,' recalling thus the supposed origin of fat from mitochondria as described by several authors. I would suggest that the 'oil droplets' are actually droplets of acrosomal material which are derived from the Golgi bodies (= vesicular bodies) in accordance with the usual procedure.

It is useless to follow the matter further at this time, since Casteel does not give any details concerning the fate of the droplets, while the Golgi bodies either degenerate or fade out through loss of staining capacity. In the adult sperm, even though it is a long rod-like affair, there seem to be no possible homologies with the head and tail regions of the flagellate sperm.

#### CONCLUSION

To summarize the results of this examination of the formation of non-flagellate sperms, it appears that:

1. The substance, which in practically all examined cases has for years been a matter of ignorance and confusion, is really the homologue of the acrosomal material in flagellate sperms, this material being rigorously defined by its derivation from the Golgi complex.

2. The term acrosome is completely inappropriate and by its implications has for many years obscured the true nature of the composition and function of the material in question. It should be renamed at the first opportunity, after its function has been more certainly determined.

3. The efforts to homologize the regions of the flagellate sperm with the parts of a non-flagellate sperm, presumably derived by a process of foreshortening, have proved a complete failure. The non-flagellate types of sperm cannot be structurally explained on the basis of comparison with each other or with flagellate types, but we can homologize their components with the same constituents in all kinds of sperms.

4. The facts concerning the formation of the acrosomal material have long been misunderstood because in so many of the non-flagellate sperms its appearance is reflected back into the primary spermatocyte, thus apparently affording time and materials for its production in unusually large amount.

#### THE FUNCTION OF THE ACROSOME

The function, or at least the fate, of the various parts of the sperm, mentioned in the earlier statement of rules of sperm formation, has been made out with fair certainty for everything except the acrosome. The nucleus is known to collaborate with that of the egg in the formation of the first cleavage figure. A centriole derivative very probably initiates the formation of the first asters and spindle for cleavage. The mitochondria often enter the egg and in some cases seem to become established in the cytoplasm (nematodes), while in others they behave more like an inert, foreign substance (echinoderms). The undifferentiated cytoplasm (apparently including the limiting membrane) gradually merges with the cytoplasm of the egg, as has been beautifully demonstrated by Meves ('11) in *Ascaris*. Concerning the acrosome very little, indeed, is known, and I have recently written (Bowen, '24 b): "The one point upon which we are still quite ignorant is the fate of the acrosome (morphologically speaking) in the egg after the sperm has once penetrated its cortex." Lillie ('12) has observed in *Nereis* that the acrosome becomes broken up into several granular fragments which eventually disappear in the egg cytoplasm. Beyond this brief statement, the fate of the acrosome in the egg is unknown.

What we require to solve this problem, as well as that of the functional significance of the acrosome, is obviously a sperm in which the acrosome is so disproportionately big that it can be followed with certainty into the egg and its every movement traced in detail. It is accordingly a matter of extraordinary interest that, granting the validity of the argument contained in the preceding pages, just such an

acrosome has been known for many years and its fate in the egg is known in considerable detail. The sperm in question is that of *Ascaris*, the refringent body of which I believe to be the homologue of the so-called acrosome of flagellate sperms. If, therefore, we discover the fate and function of the refringent body in the *Ascaris* egg, we may very well be provided with a basis for drawing some general conclusions of far-reaching import.

The behavior of the refringent body in the egg of *Ascaris* has been observed by many workers; Meves ('11) has given perhaps the best account of it. His work was done on eggs preserved for the mitochondria with osmic fixatives, and hence in all probability well suited for preserving the refringent body. After staining in acid fuchsin and picric acid, the cytoplasm of the sperm stains a distinctive yellow-brown, while the refringent body is deep red. Meves found that this body entered the egg in the intact sperm, and during the long interval preceding the formation of the first cleavage spindle, it becomes progressively smaller. Eventually it disappears completely, sometimes leaving behind for a time a clear space, which marks the place of the last remnant to disappear. In other words, the refringent body is gradually dissolved and its substance liberated into the cytoplasm of the egg. Other observers have described the breaking up of the refringent body into several pieces which subsequently disappear. This history agrees with that described for *Nereis* by Lillie. We may, I think, tentatively conclude that the acrosome normally enters the egg with the sperm, and during the earlier phases of fertilization goes gradually into solution in the egg cytoplasm.

The function of this material thus released into the cytoplasm is problematical, but I have recently suggested (Bowen, '24 b) that, "The acrosome is essentially a secretory product the principal function of which is to initiate the physico-chemical reactions of fertilization." In any event, it is obvious that if the acrosome has an active rôle in fertilization, it must enter the egg. There are, however, several observa-

tions to the effect that the refringent body of the *Ascaris* sperm is not necessarily present in the sperm when it enters the egg, and accordingly is not essential for fertilization. On the contrary, other workers claim that this is not the case, and that only a complete spermatozoon can accomplish fertilization. In view of the fact that the female genital tract often contains degenerating sperms from which the refringent body is absent, and that in any event the fixation of the eggs is often technically defective, it seems very probable that abnormalities might readily occur leading to erroneous conclusions. Certainly, the general rule is that the sperm carries the refringent body into the egg, and there is a reasonable probability that only such complete sperms can produce normal fertilization and development.

With these uncertainties still to be resolved, it is perhaps hazardous to venture on a discussion of the function of the acrosome based on the conditions in *Ascaris*. But I cannot refrain, in concluding, from calling attention to the fact that, coincident with the dissolution of the refringent body, very extensive changes occur in the cytoplasm of the egg. These eventuate in the extrusion of a considerable amount of material from the egg, which forms a shell that encloses the egg in one of the most remarkable membranes of its kind. That there is some relation between the extensive cytoplasmic activity which produces this unusual membrane, on the one hand, and the remarkably large quantity of 'acrosomal' material, on the other, is an hypothesis too attractive to be avoided.<sup>7</sup> And in such a possibility I find the basis for some tangible evidence bearing on the function of the acrosome. In any event, if these suggestions prove acceptable, we have in the *Ascaris* sperm a remarkable possibility for critical experimental attacks on the microchemistry of the acrosome.

The thesis which I have in this paper tried to develop places the whole problem of the acrosome for the first time

<sup>7</sup> Compare also the findings of Goodrich ('20) on membrane formation and maturation in *Nereis*, the sperm (i.e., the acrosome-bearing end) having been allowed to come in contact with the egg and then removed at once.



on a broad comparative basis, and in my view suggests a path along which we may hope to reach a final solution. Whether true or false, it at least seems to recommend itself for the facility with which it brings under a common point of view an immense series of otherwise isolated and puzzling observations.

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# THE RELATIVE POSTNATAL GROWTH OF THE SYSTEMS AND ORGANS OF THE CHICKEN <sup>1</sup>

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## TEN CHARTS

The growth of the body, systems and organs of the single-comb White Leghorn chicken has been described in a recent paper, but a comparison of the growth of the various parts with reference to the entire body growth was not given, and so this little study has been undertaken to show the relative changes in the growth of the systems and organs between hatching and maturity.

## MATERIAL AND METHODS

The data on which this study is based are taken from a previous paper (Latimer, '24) in which is reported the growth of 100 single-comb White Leghorn chickens, raised at the University of Minnesota and ranging in age from day of hatching to 300 days of age, with three specimens about one year old and three about two years old. The details of the study of the growth in gross body weight and of the methods of making the autopsies are given in the earlier report and will not be included here. All of the weights in grams given in this paper were determined by using the empirical formulae given in the earlier report and the percentage values were secured by inspection of the percentage curves used in the preparation of the charts of the earlier paper. The percentage weight in all cases is the weight of

<sup>1</sup>Studies from the Zoölogical and Anatomical Laboratories of the University of Nebraska, no. 141.



the system or organ reduced to a percentage of the net body weight, or the weight of the chicken immediately after killing minus the weight of the contents of the digestive tube. The method of determining the ratios of the percentage weights is the same as that used by Donaldson ('23) in his study of the albino rat. I wish to express my thanks to the Department of Anatomy of the University of Minnesota for making possible this series of studies on the growth of the chicken.

#### TOTAL INCREASE IN WEIGHT

The total increase in weight from hatching to maturity is not the same for the various systems and organs of the chicken, but ranges from an increase of about sevenfold for the central nervous system to a maximum of 214 times for the pancreas. The growth increases for the various parts are summarized in table 1.

The first column of table 1 gives the name of the organ or system; the second column, the hatching weight in grams, and the third column gives the weight at maturity. The last column gives the ratios of the weight at maturity to the hatching weight. These ratios greatly facilitate comparison of the growth in weight of the various parts. A study of this table will show that the integument and the gross body weight each increase a little over 70 times, and that there are twenty parts increasing less than 70 times and but three which increase more than the gross body weight. As is to be expected, those parts and organs which have a precocious development increase less in postnatal life. The central nervous system and the eyeballs increase but 6.6 times from hatching to maturity, followed by the hypophysis and the head of the females. A study of the postnatal growth of the central nervous system of the domestic fowl (Latimer, '25) shows that the brain and the spinal cord do not increase an equal amount between hatching and maturity, for the cord increases 19 times during this period and the brain increases but fourfold. There is a difference in the total increase in the parts of the brain, although not so great a difference as that between the brain and the cord.

The head of the male chickens increases over twice as much as does the head of the females, but this is due largely to the heavy comb and wattles of the male fowls, the comb, wattles, and ear lobes being included in the weight of the head of both sexes.

TABLE 1  
*Increase in weight of the various organs and systems*

|                         | HATCHING<br>WEIGHT IN<br>GRAMS | WEIGHT AT<br>MATURITY | INCREASE<br>TIMES | MAXIMUM<br>INCREASE |
|-------------------------|--------------------------------|-----------------------|-------------------|---------------------|
| Nervous system.....     | 1.00                           | 6.58                  | 6.6               |                     |
| Eyeballs.....           | 0.80                           | 5.28                  | 6.6               |                     |
| Hypophysis .....        | 0.0021                         | 0.0155                | 7.4               |                     |
| Head (female).....      | 4.0                            | 53.2                  | 13.3              |                     |
| Stomach.....            | 0.40                           | 5.78                  | 14.5              |                     |
| Digestive tube .....    | 4.67                           | 107.3                 | 23.0              |                     |
| Gizzard.....            | 2.0                            | 50.2                  | 25.1              |                     |
| Intestines .....        | 1.87                           | 50.6                  | 27.0              |                     |
| Head (male) .....       | 4.0                            | 112.8                 | 28.2              |                     |
| Viscera.....            | 6.89                           | 194.7                 | 28.3              |                     |
| Suprenals .....         | 0.008                          | 0.254                 | 31.8              |                     |
| Liver.....              | 1.5                            | 50.9                  | 33.9              |                     |
| Kidneys.....            | 0.4                            | 15.3                  | 38.2              |                     |
| Skeleton (female).....  | 5.2                            | 201.2                 | 38.7              |                     |
| Skeleton (male).....    | 5.2                            | 254.1                 | 48.9              |                     |
| Heart .....             | 0.25                           | 13.7                  | 54.6              |                     |
| Spleen .....            | 0.04                           | 2.73                  | 68.3              |                     |
| Gross body weight ..... | 37.0                           | 2600.0                | 70.3              |                     |
| Integument.....         | 3.1                            | 217.4                 | 70.3              |                     |
| Thyroid .....           | 0.0025                         | 0.284                 | 113.6             |                     |
| Musculature .....       | 8.2                            | 1257.3                | 153.3             |                     |
| Pancreas .....          | 0.02                           | 4.28                  | 214.0             |                     |
| Thymus .....            | 0.10                           | 0.75                  | 7.5               | 60.6                |
| Feathers (female).....  | 1.8                            | 87.6                  | 48.1              | 61.3                |
| Feathers (male) .....   | 1.8                            | 118.1                 | 64.9              | 81.6                |

The viscera increase a little over 28 times, or but a little more than the head of the male. The weights of the viscera as given in this table are the sums of the weights of the various organs, a list of which is given in table 2, which gives the ratios of the percentage weights. The lungs are not

included, unfortunately, for after 1200 grams of gross body weight they were so variable that no formula was deduced and no curve drawn. The weights of the two lungs and the trachea together for each chicken are shown plotted on the chart in figure 19 of the previous report.

The male skeleton increases more than the female skeleton, or 49 times for the male and but 39 times for the female skeleton. The male skeleton is followed by the heart, which increases 55 times and the spleen which increases 68 times, or nearly as much as the total body weight increase.

The thyroid, the musculature, and the pancreas each increase more than the total body weight increase, the values are respectively, 114, 153, and 214 times. The thyroid and the pancreas each form but a relatively small part of the total body weight, but the skeletal muscles increase from 22 per cent of the net body weight at time of hatching to 48 per cent in the adult fowl. An increase of over 150 times in the absolute weight of a part forming so large a percentage of the entire body weight would naturally account for the larger number of organs increasing less than 70 times, or the increase for the entire body from hatching to maturity. The large increase in the thyroid weight is due in part, at least, to the increased amount of colloid and the increase of 214 times for the pancreas may be due to some extent to the difficulty in removing all of the gland in the newly hatched chicks.

The increase of the thymus and the feathers for the male and the female are listed as the last three organs in table 1, for although they increase, respectively, but 8, 48, and 65 times their hatching weights at maturity, they do have maxima much higher than these figures earlier in their growth. At its maximum the thymus is 61 times its hatching weight, or a little over 8 times the ratio at maturity. The feathers of the female at their maximum are 1.3 times the ratio at maturity and the maximum for the male plumage is a little over 1.2 times the ratio at maturity.

## RELATIVE PERCENTAGE WEIGHTS

The weights of the various organs and systems reduced to percentages of the net body weight are shown plotted against the gross body weight in the previous paper.

The individual percentages were plotted on a preliminary chart and a curve was drawn by inspection through the

TABLE 2

*Relative percentage weights of the sum of the viscera and of each individual organ*

| GROSS BODY<br>WEIGHT IN<br>GRAMS | VISCERA<br>(SUM OF<br>ORGANS) | HYPOPHYSIS | STOMACH | DIGESTIVE<br>TRACT | GIZZARD | INTESTINE | SUPRARENALS |
|----------------------------------|-------------------------------|------------|---------|--------------------|---------|-----------|-------------|
| 37                               | 1.00                          | 1.00       | 1.00    | 1.00               | 1.00    | 1.00      | 1.00        |
| 100                              | 1.01                          | 0.57       | 0.87    | 0.93               | 0.94    | 1.13      | 0.81        |
| 300                              | 0.89                          | 0.24       | 0.64    | 0.76               | 0.77    | 0.88      | 0.63        |
| 500                              | 0.81                          | 0.18       | 0.55    | 0.68               | 0.66    | 0.82      | 0.52        |
| 700                              | 0.76                          | 0.16       | 0.49    | 0.64               | 0.60    | 0.78      | 0.47        |
| 900                              | 0.71                          | 0.13       | 0.42    | 0.60               | 0.57    | 0.73      | 0.45        |
| 1100                             | 0.66                          | 0.11       | 0.38    | 0.55               | 0.54    | 0.67      | 0.45        |
| 1300                             | 0.61                          | 0.092      | 0.33    | 0.51               | 0.51    | 0.62      | 0.44        |
| 1500                             | 0.56                          | 0.079      | 0.30    | 0.47               | 0.48    | 0.57      | 0.41        |
| 1700                             | 0.51                          | 0.079      | 0.26    | 0.42               | 0.45    | 0.50      | 0.39        |
| 1900                             | 0.47                          | 0.072      | 0.23    | 0.38               | 0.40    | 0.45      | 0.37        |
| 2100                             | 0.44                          | 0.072      | 0.22    | 0.35               | 0.37    | 0.40      | 0.36        |
| 2300                             | 0.41                          | 0.072      | 0.20    | 0.33               | 0.34    | 0.37      | 0.35        |
| 2600                             | 0.39                          | 0.072      | 0.18    | 0.30               | 0.31    | 0.32      | 0.35        |

| GROSS BODY<br>WEIGHT IN<br>GRAMS | LIVER | KIDNEYS | HEART | SPLEEN | THYROID | PANCREAS | THYMUS |
|----------------------------------|-------|---------|-------|--------|---------|----------|--------|
| 37                               | 1.00  | 1.00    | 1.00  | 1.00   | 1.00    | 1.00     | 1.00   |
| 100                              | 1.07  | 1.85    | 0.97  | 2.50   | 0.92    | 1.72     | 1.05   |
| 300                              | 1.01  | 1.96    | 0.84  | 4.17   | 0.72    | 1.50     | 1.27   |
| 500                              | 0.92  | 1.81    | 0.75  | 3.83   | 0.76    | 1.25     | 1.47   |
| 700                              | 0.88  | 1.60    | 0.69  | 3.34   | 0.89    | 1.16     | 1.62   |
| 900                              | 0.84  | 1.46    | 0.63  | 3.50   | 0.98    | 1.02     | 1.71   |
| 1100                             | 0.80  | 1.31    | 0.60  | 3.67   | 1.01    | 0.92     | 1.78   |
| 1300                             | 0.76  | 1.15    | 0.57  | 3.17   | 1.02    | 0.83     | 1.45   |
| 1500                             | 0.71  | 1.06    | 0.58  | 2.67   | 1.03    | 0.75     | 1.00   |
| 1700                             | 0.67  | 0.99    | 0.60  | 2.42   | 1.04    | 0.69     | 0.64   |
| 1900                             | 0.61  | 0.91    | 0.65  | 2.17   | 1.07    | 0.64     | 0.44   |
| 2100                             | 0.57  | 0.86    | 0.66  | 2.08   | 1.15    | 0.58     | 0.31   |
| 2300                             | 0.53  | 0.80    | 0.66  | 2.00   | * **    | 0.56     | 0.20   |
| 2600                             | * **  | 0.73    | 0.66  | 1.83   | * **    | 0.56     | 0.09   |



double-weighted medians, or the averages for both the percentage weight of the organ and the gross body weight for each 200 grams' increase in body weight, and this curve without the individual cases is shown as the lighter line in the charts in the previous paper. The percentage values read from these curves were used in computing the ratios for this report. In many cases the original data were consulted in verification of the percentages. The percentage weight at hatching was taken as unity and the ratios of the percentages for each organ at 100 grams and for each 100 grams' increase in gross body weight thereafter were determined. Figures 1 to 10 show these ratios graphically and tables 2 and 3 give the ratios for hatching, 100 grams and each 200 grams' increase in gross body weight thereafter. This treatment makes a comparison of the changes in the percentages very much easier than a direct inspection of the percentage curves.

The ratios for the viscera and the individual organs which are listed as viscera are given in table 2. The sum of the percentages of the individual organs given in this table were added, and these results were treated as were the percentages of the individual organs, and these ratios are given in the second column, the gross body weights being given in the first column. The ratios for the digestive tube, given in the fifth column, are the ratios for the sums of the parts of the digestive tube, namely, the stomach or proventriculus, the gizzard and the intestines, esophagus, and the crop all weighed together. These parts are given separately as well as their sum, and all the ratios are for the empty digestive tube or its parts. To complete the list, the lungs and trachea should be included, but, as previously stated, they were so variable in weight that it did not seem wise to include them. There were no apparent pathological lungs in the group—at least, no pathological specimens were noticed in making the autopsies.

The ten viscera (male and female together) listed in table 2 form 21.4 per cent of the net body weight at time of hatching.

This rises to 21.7 per cent at 100 grams of body weight and then slowly decreases to 8.3 per cent in the adult. These values of course do not include the percentage weight of the lungs and the submaxillary which are included by Donaldson ('23) in the percentage weights of the viscera of the albino rat. The viscera of the male albino rat form 10.9 per cent of the body weight at birth, 19.2 per cent at 25 grams of body weight, and then decrease to 10.5 per cent in the adult. These figures show that at birth the percentage weights of the vis-

TABLE 3  
*Relative percentage weights of the parts and systems*

| GROSS BODY<br>WEIGHT IN<br>GRAMS | CENTRAL<br>NERVOUS<br>SYSTEM | HEAD<br>(FEMALE) | HEAD<br>(MALE) | SKELETON<br>(FEMALE) | SKELETON<br>(MALE) | MUSCLES | FEATHERS<br>(FEMALES) | FEATHERS<br>(MALE) |
|----------------------------------|------------------------------|------------------|----------------|----------------------|--------------------|---------|-----------------------|--------------------|
| 37                               | 1.00                         | 1.00             | 1.00           | 1.00                 | 1.00               | 1.00    | 1.00                  | 1.00               |
| 100                              | 0.63                         | 0.66             | 0.66           | 1.07                 | 1.07               | 1.41    | 1.41                  | 1.41               |
| 300                              | 0.33                         | 0.41             | 0.41           | 1.07                 | 1.07               | 1.75    | 2.00                  | 2.00               |
| 500                              | 0.25                         | 0.33             | 0.41           | 1.09                 | 1.09               | 1.87    | 2.16                  | 2.16               |
| 700                              | 0.20                         | 0.29             | 0.37           | 1.05                 | 1.05               | 1.95    | 2.24                  | 2.24               |
| 900                              | 0.17                         | 0.25             | 0.33           | 1.02                 | 1.02               | 2.00    | 2.40                  | 2.40               |
| 1100                             | 0.15                         | 0.23             | 0.31           | 0.89                 | 1.02               | 2.04    | 2.65                  | 2.65               |
| 1300                             | 0.14                         | 0.21             | 0.29           | 0.76                 | 1.01               | 2.07    | 2.54                  | 2.59               |
| 1500                             | 0.12                         | 0.21             | 0.30           | 0.68                 | 0.96               | 2.10    | 2.00                  | 2.54               |
| 1700                             | 0.11                         | 0.19             | 0.31           | 0.63                 | 0.89               | 2.12    | 1.59                  | 2.40               |
| 1900                             | 0.11                         | 0.18             | 0.33           | 0.60                 | 0.82               | 2.14    | 1.38                  | 2.16               |
| 2100                             | 0.097                        | 0.18             | 0.34           | 0.58                 | 0.76               | 2.16    | 1.16                  | 1.84               |
| 2300                             | 0.090                        | 0.18             | 0.37           | 0.56                 | 0.74               | 2.17    |                       | 1.57               |
| 2600                             | 0.081                        | 0.18             | 0.40           | 0.55                 | 0.69               | 2.19    |                       |                    |

cera of the chicken (excluding the lungs) are just a little less than twice as great as those of the viscera in the rat. The maximum percentage weights are more nearly alike, for the chicken viscera are now but slightly heavier than the viscera of the rat. At maturity the viscera of the rat form a larger percentage of the body weight than do the viscera of the chicken.

Similar data are given in table 3 for the systems and parts of the body not listed as viscera. The central nervous system is given all together here, as a study of the growth of the

parts of the brain and of the spinal cord of the chicken has been published (Latimer, '25).

As stated above, the values used in determining the ratios given in tables 2 and 3 and plotted in figures 1 to 10 are taken from the curves drawn by inspection. There are, of course, individual variations in the percentage weights and these are referred to in the earlier paper, the curve merely giving the

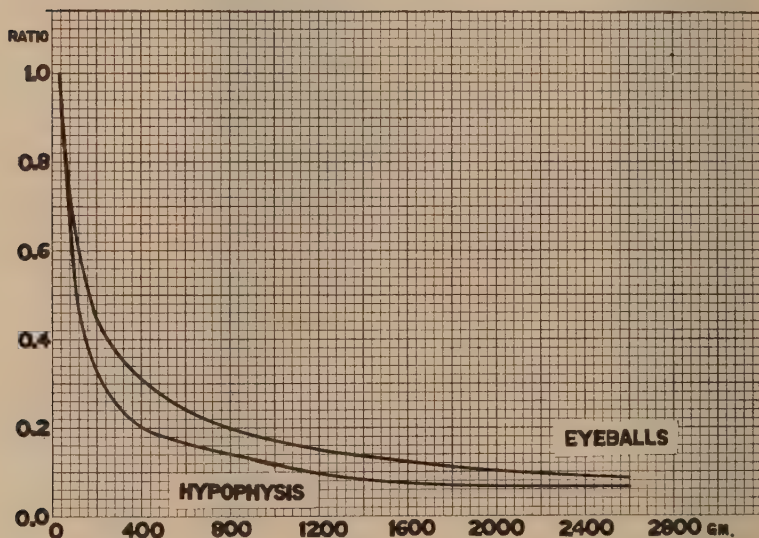


Fig. 1 The percentage weights of the eyeballs (upper curve) and the hypophysis (lower curve) reduced to ratios of the percentage weights at hatching plotted against gross body weight in grams.

median values for each body weight. A careful study of the distribution of the cases for the digestive tube shows that the percentage growth of this part increases for the first six days and that there is also a similar very brief rise in the percentage values of the liver (fig. 5). The curve for the entire digestive tube is not shown. It follows the curve for the gizzard (fig. 4) very closely, and so it was not drawn on the chart. The data are given in table 2.

It will readily be seen by a study of the curves in figures 1 to 10 that there are four types of curves. There are, first of all and found in the largest number of cases, the curves which decline from hatching to maturity. Included in this type are the central nervous system (brain and spinal cord), eyeballs, hypophysis, glandular stomach or proventriculus, entire digestive tract, gizzard, head (both male and female),

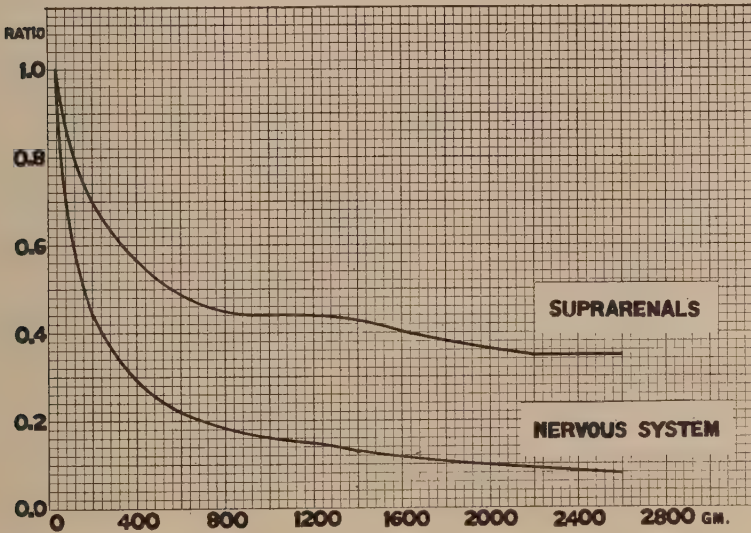


Fig. 2 The percentage weights of the suprarenals (upper curve) and the entire central nervous system (lower curve) reduced to ratios of the percentage weight at hatching plotted against gross body weight in grams.

suprarenals, and the heart. While the heart is listed in this group, it does show a rise toward the latter part of the period, for after a minimum ratio of 0.57 at a gross body weight of 1300 grams the ratio slowly rises to 0.66 at 2100 grams and remains at this level up to maturity.

In the second group of curves are those which rise to a maximum after hatching and then later decrease to a ratio less than unity, or to a smaller percentage of the net body weight than at hatching. This group includes the following:



pancreas, liver, intestines (including esophagus and crop), viscera (sum of organs listed in table 2), kidneys, ligamentous skeleton (both male and female), and the thymus.

In the third group are listed the two organs which rise to a maximum after hatching and then decrease slightly, but whose ratio remains greater than unity, or which form a larger percentage of the net body weight even at maturity than they do at time of hatching. The feathers and the spleen

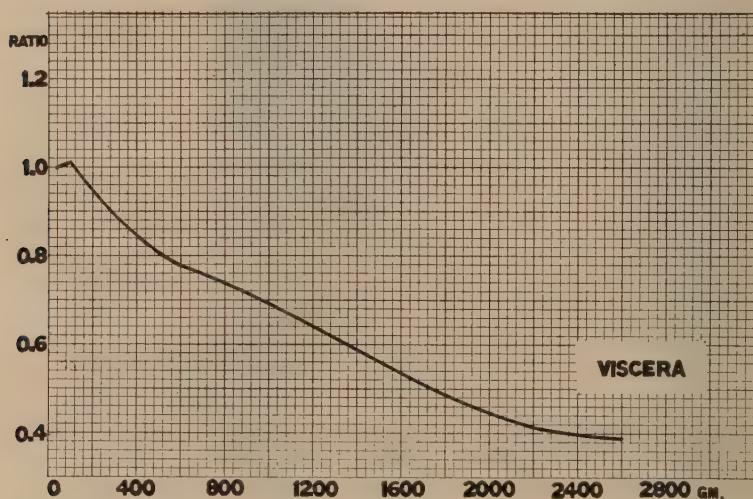


Fig. 3 The ratios of the viscera (listed in table 2) plotted against gross body weight in grams.

are the two curves included in this group. Figure 10 shows that the female plumage returns to nearly the same percentage of the net body weight at maturity as it formed at hatching. The curve showing the ratios of the percentage weights of the spleen (fig. 8) shows two maxima, the first which is also the most marked at 300 grams of gross body weight and the second at 1000 or 1100 grams of gross body weight.

The fourth group, or those parts with a ratio greater than unity or a larger percentage of the net body weight at maturity than at time of hatching, includes the thyroid and

the musculature. The thyroid (fig. 7) drops to a minimum ratio of 0.72 at 300 gráms of gross body weight, but rises thereafter, attaining a maximum ratio of 1.15 at 2100 grams of gross body weight. After this body weight the thyroid was so variable that it seemed best to carry the curve to this point only.

#### PERIODS OF MAXIMUM PERCENTAGE WEIGHTS

Table 4 gives the gross body weights at which the maximum percentage ratios occur for the various organs. There are

TABLE 4  
*Body weights at which the organs and systems attain their maximum percentage weight*

| BODY WEIGHT          | ORGANS                 | BODY WEIGHT | ORGANS      |
|----------------------|------------------------|-------------|-------------|
| Hatching or 37 grams | Nervous system         | 300 grams   | Kidneys     |
|                      | Hypophysis             |             | Spleen      |
|                      | Eyeballs               | 500 grams   | Skeleton    |
|                      | Head (male and female) |             |             |
|                      | Heart                  | 1100 grams  | Thymus      |
|                      | Suprarenals            |             | Feathers    |
|                      | Gizzard                | 2100 grams  | Thyroid     |
|                      | Stomach                |             |             |
| 100 grams            | Digestive tube         | 2600 grams  | Musculature |
|                      | Pancreas               |             |             |
|                      | Liver                  |             |             |
|                      | Intestines             |             |             |
|                      | Viscera                |             |             |

nine organs and systems of the chicken which have this maximum percentage at time of hatching. The brain and the spinal cord are here taken together, but each, as previously shown, has its maximum percentage of the net body weight at time of hatching. This initial maximum is found in the case of the eyeballs, the hypophysis, the head, the heart, and the entire digestive tube. The maximum for the digestive tube, as described above, really comes on the sixth day. A comparison of this table with table 1 given by Donaldson ('23) shows that of these nine entries in table 4, but one,

the hypophysis, forms its maximum percentage at birth in the albino rat. Donaldson gives the lungs, the hypophysis, and the thyroid as forming their maximum percentage weight at birth in the rat. Examination of the percentages of the chick lungs shows that they, too, might be included in this list, for without doubt their maximum percentage weight occurs at

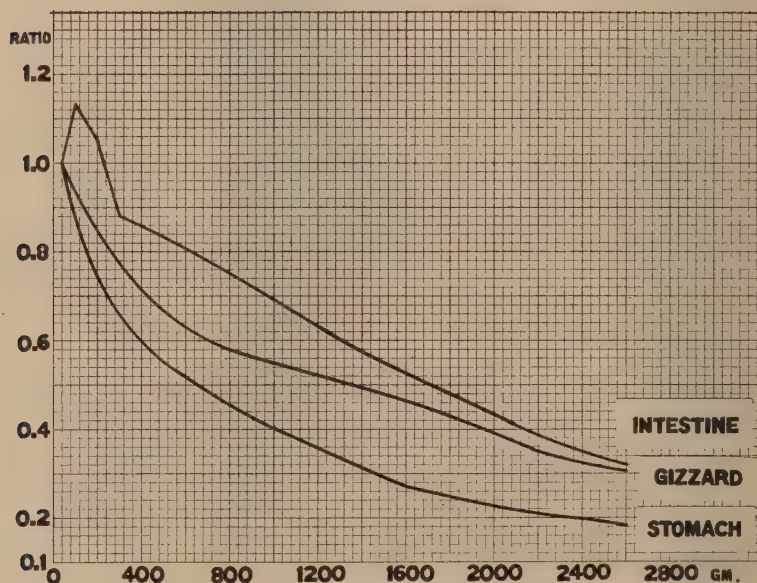


Fig. 4 The ratios of the parts of the digestive tract, intestine (upper curve), gizzard (middle curve), and stomach (lowest curve), plotted against gross body weight in grams. The curve for the entire empty digestive tract (not shown) follows very closely the curve for the gizzard.

time of hatching. This would make two organs with a common maximum percentage weight at birth in the chick and in the albino rat, namely, the lungs and the hypophysis. The percentage of the thyroid does decrease for a time in the chick (fig. 7), but it later increases to a greater relative weight at 2100 grams. The maximum relative weight of the heart of the chick at hatching may be explained by its greater activity compared with that of the newborn rat, thus necessitat-

ing a much more effective circulation in the chick. Joseph ('08) has suggested that the activity of an animal is directly correlated with its heart size. The precocious development of the digestive tube in the chick may be explained by the difference in the diet—the rat is nourished by the maternal

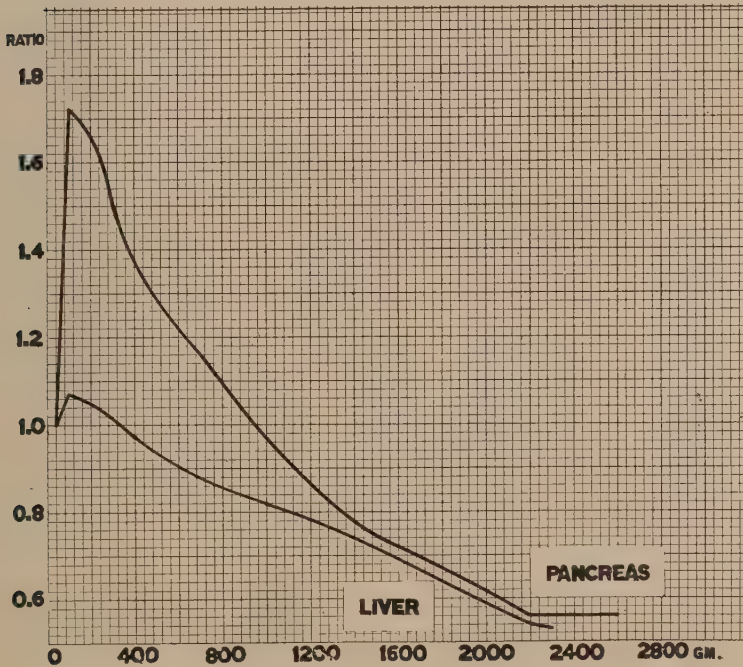


Fig. 5 The ratios of the pancreas (upper curve) and the liver below plotted against gross body weight in grams.

milk, while the chick must eat nearly the same diet as the adult, and hence it needs a digestive system fully able to care for this complex food. Donaldson ('23) finds the maximum percentage weight of the digestive tract of the rat at a body weight of 31.1 grams, or at about six times the birth weight.

Jackson ('13) finds the head of the albino rat reaching its maximum relative weight at seven days, but in the chick this



maximum occurs at hatching. This may be explained by the better development of the eyes and central nervous system in the chick, again illustrating the better development of the chick at time of hatching compared with the albino rat,

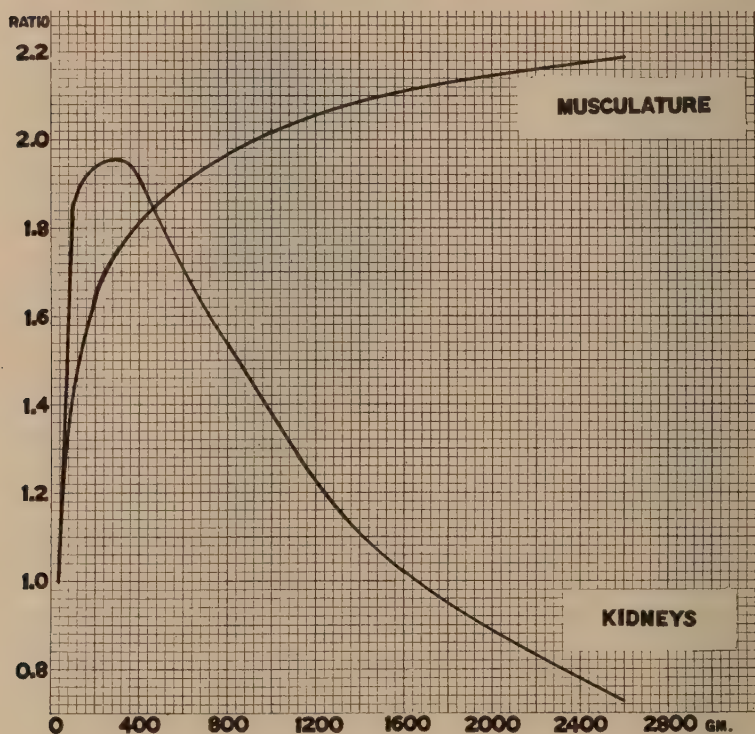


Fig. 6 The ratios of the percentage weights of the musculature and the kidneys plotted against gross body weight in grams. The curve which rises continuously is that of the musculature.

which is born blind and dependent upon maternal protection and food supply, while the chick, if supplied with a source of artificial heat for part of the time, will get along fairly well. The suprarenals of the rat reach their maximum percentage weight when the body is about five times its birth weight, but in the chick they are relatively largest at hatching.

The pancreas, liver, intestines (including esophagus and crop), and the sum of all the viscera attain their maximum at 100 grams of gross body weight.

The spleen and the kidneys do not attain their maximum percentage until a body weight of 300 grams is reached, or at about forty days of age. This retardation in the attainment of the maximum percentage weight of the kidneys may be due possibly to the embryonic elimination of the nitrogenous waste through the allantois and the fact that the kidneys do not function very extensively until after hatching. Figure

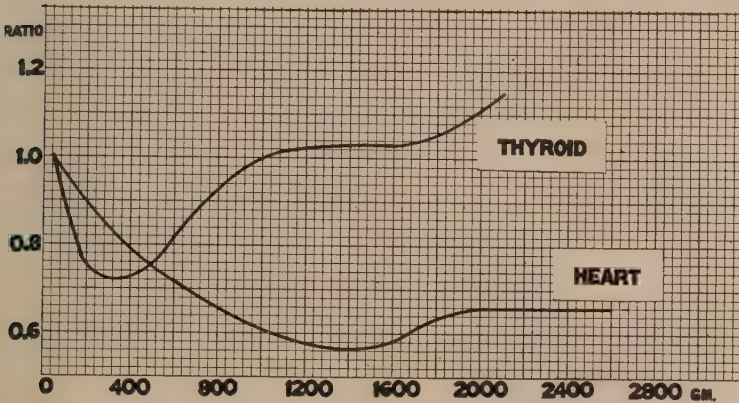


Fig. 7 The ratios of the thyroid and the heart plotted against gross body weight in grams.

8, which shows the graphic representation of the ratios for the spleen, shows two maxima, one which is the greater, at 300 grams and another at 1000 or 1100 grams of gross body weight.

The skeleton reaches its maximum percentage weight at 500 grams, or between sixty and seventy days of age or slightly after the end of the period of most rapid percentage increase of the skeletal musculature. Jackson and Lowrey ('12) report a slight increase in the percentage weight of the skeleton of the albino rat during the first week of postnatal life, and then, following this maximum, the percentage

weights decrease to maturity. The musculature does not reach its maximum until maturity, but its period of most rapid relative growth is passed by the time the body has attained a weight of about 300 grams. It is interesting to note that the maximum percentage weight of the cerebellum (Latimer, '25) also occurs at from 400 to 600 grams of gross body weight, suggesting the similarity in relative development of the skeleton, musculature, and the cerebellum.

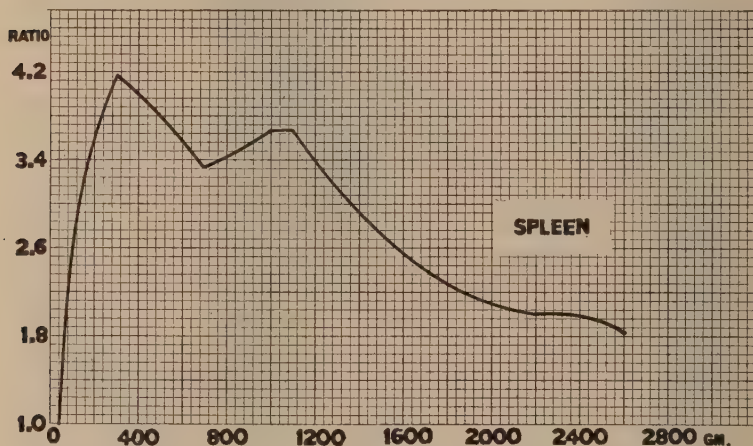


Fig. 8 The ratios of the percentage weights of the spleen plotted against gross body weight in grams. The scale of the ordinates here is one-half that of the other figures.

The thymus and the feathers present similar ratio curves, except that there is a sex difference in the percentages of the feathers and none in the case of the thymus. Both reach their maximum relative weight at 1100 grams, or at the same time as the second maximum for the spleen. Riddle ('25), in a recent publication, has described a growth curve of the thymus in pigeons similar to that of the chicken.

The relative weights of the thyroid are shown in figure 7 from hatching to 2100 grams of gross body weight only, for after this time the weights of the individual thyroids varied to such an extent that it seemed best to exclude these weights

as unreliable. Some of the heavier adult thyroids may have been pathological, and so, while the individual weights are indicated in figure 21 of the earlier paper, the formula and the data are carried to 2100 grams of gross body weight only.

The musculature increases up to maturity. Some of this increase in the older chickens may be due to an increased amount of fat stored within the muscle and which could not

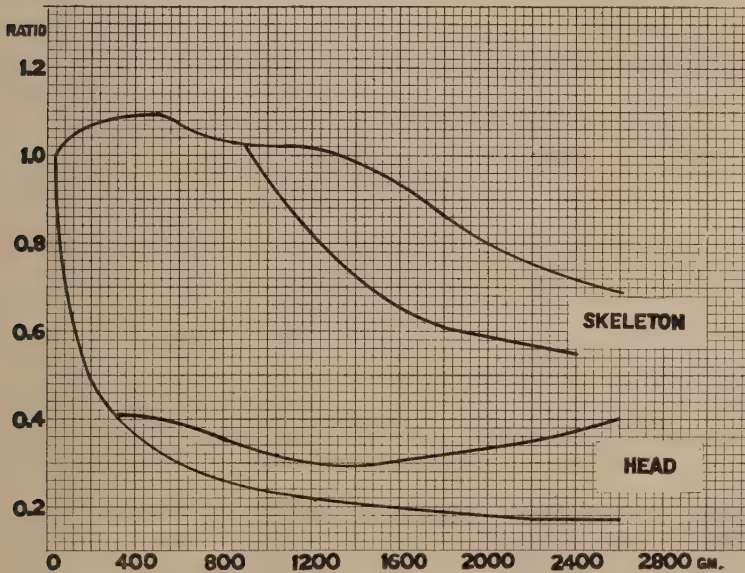


Fig. 9 The ratios of the skeleton (above) and the head (below) plotted against gross body weight in grams. Both curves show a sex difference in the latter part, the upper part of each represents the ratios for the males and the lower, the females.

be separated mechanically from the muscle tissue. Jackson and Lowrey ('12) find the musculature of the newborn albino rat forming 24.4 per cent of the net body weight. In the one-week-old rat they find this percentage reduced to 22.8 per cent, and following this an increase to 45.4 per cent in the adult. The chicken musculature increases continuously from 22 per cent at time of hatching to 48 per cent at maturity.



## THE LIFE-CYCLE IN THE CHICKEN AND IN MAN

Donaldson ('18) has shown that from birth to old age man develops one-thirtieth as fast as the albino rat, but the growth of the chicken does not show a constant ratio to the growth of man. The comparison of the growth of the chicken and of man for six periods is shown in table 5. During the first two periods, or the embryonic period and the time in which the birth weight is doubled, the chicken is growing 13 times faster than man. After this the growth is accelerated in the chick, as compared with the human growth, for the chick triples its hatching weight in twenty-three days and man in one year, or one-sixteenth the time required by man.

TABLE 5  
*Growth of the chicken compared with that of man*

| PERIODS                        | CHICKEN  | MAN      | RATIO |
|--------------------------------|----------|----------|-------|
| Embryonic .....                | 21 days  | 280 days | 1/13  |
| Double birth weight .....      | 14 days  | 180 days | 1/13  |
| Three times birth weight ..... | 23 days  | 365 days | 1/16  |
| Puberty (female) .....         | 6 months | 14 years | 1/30  |
| Growth in length ceases .....  | 8 months | 20 years | 1/30  |
| Old age .....                  | 7 years  | 90 years | 1/13  |

This acceleration is further increased in the comparison of the times of puberty for the female of both species and for the time of the cessation of increase in body length. In both of these the ratio is one-thirtieth, or these two periods are reached by the chick in one-thirtieth the time required by man. The time of puberty is given as six months for the females only, as this period can be more easily determined in the female fowl. This age may be a little too early, and yet in both the chick and in man there is a wide variation in the age of puberty, and so both are merely approximations.

Schneider and Dunn ('24), in their study of the lengths of the bones of the fowl, conclude that the bone length is completed by eight months of age in the Leghorn. The skeletons of the present series of chickens are being studied (Latimer,

'23) and the ossifications of the long bones are all completed before the two hundredth day, so the cessation of growth in length is placed at eight months for the chick.

The last period, or the age of the chicken comparable to ninety years in man, is more difficult to determine, for

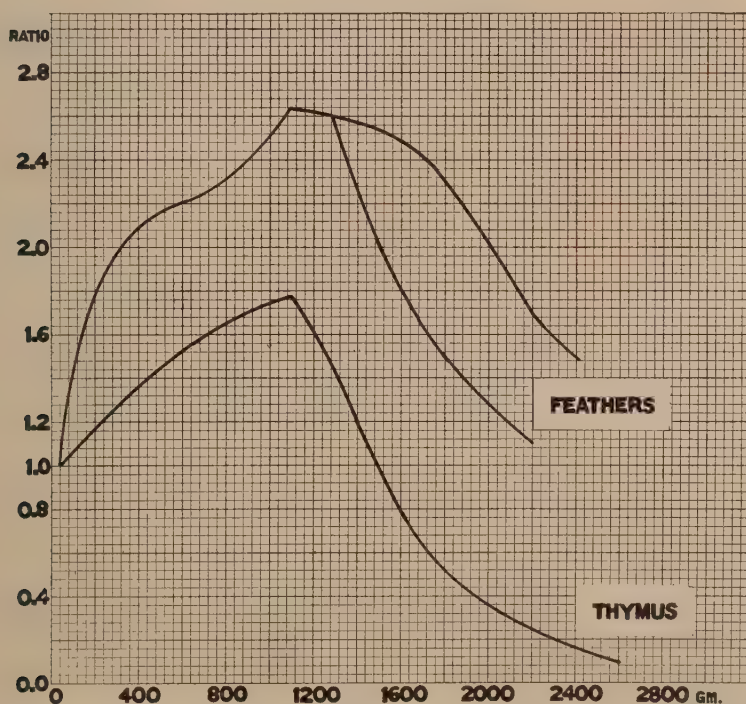


Fig. 10 The ratios of the feathers (above) and the thymus (lower curve) plotted against gross body weight in grams. The curve for the feathers shows a sex difference after 1100 grams, the upper arm of the curve representing the ratios for the males and the lower, the females.

chickens usually do not die of old age, and so this is more or less an approximation. Seven years may be too young. I have learned of two bantam hens which lived to be ten and twelve years old, and the one which was accidentally killed at ten years layed up to nine years of age. The men in our

Poultry Husbandry Department feel that seven years is a fair estimate, although there seem to be no definite data on this point. An age greater than seven years for the chicken would only accentuate the point of the chart, namely, that the time of old age in the chicken comes back to the same ratio as the first two periods or possibly less than this ratio.

We may conclude that the chicken matures more rapidly, thus leaving a relatively longer period of maturity than is found in man. The chicken which belongs to a lower order of vertebrates thus has a shorter period of growth and a relatively longer period of mature life when compared with the growth cycle of man.

#### SUMMARY

1. The gross body weight of the chicken increases 70 times from hatching to maturity. The central nervous system, eyeballs, and hypophysis increase but about 7 times, all the viscera together increase 28 times, and but three structures, the thyroid, the musculature, and the pancreas, increase more than 70 times.

2. The percentage weights of the systems and organs reduced to ratios of the percentage weight at time of hatching fall into four groups: *a*) those parts which decrease from hatching to less than the hatching percentage at maturity—this group includes the larger number of systems and organs; *b*) those parts which rise to a maximum after hatching, but at maturity have a smaller percentage than at time of hatching; *c*) the third group includes but two organs, the feathers and the spleen, each of which rises to a maximum after hatching, but whose percentage remains greater than the hatching percentage at maturity, and, *d*) those parts which rise to their maximum at maturity.

3. Ten systems and organs in the chicken have their maximum percentage at time of hatching. This shows that more organs in the chick develop earlier than in the rat, due probably to the more active and independent life of the chick immediately after hatching.

4. A comparison of the life-cycle in the chick and in man shows that the development of the chick increases in rapidity when compared with the development of man, thus leaving a relatively longer period of mature life in the chicken.

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## THE REPAIR OF BONE

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FOURTEEN FIGURES

### INTRODUCTION

The chief object of this work is to bring before the anatomist and teacher a comprehensive statement of the successive processes concerned in the repair of bone following a particular type of injury, namely, incomplete fracture produced by a sawcut. Material collected in review of the literature on bone repair indicates that certain elements play parts which vary in degree depending upon the type of injury produced. We wish to acknowledge our indebtedness to Dr. Basil C. H. Harvey for suggesting the problem.

The problem of the growth of bone and its repair has been before us more or less constantly since 1739. Keith gives an adequate, readable, historical summary of the subject. Todd has recently emphasized the importance of the cancellous bone, and Wieder has published a long list of experiments in which he covers one factor after another. Wieder covers the entire subject with varying degrees of thoroughness, and on the whole gives a good survey of the entire field. In view of the accessibility of these papers, a long preliminary discussion is unnecessary; we will confine ourselves largely to a description of our material.

It seems well established that all parts of the bone and the periosteum are involved in varying degrees in the process of repair, but the influence of the fibrous periosteum is not clear. The rôle played by each layer will be discussed in a later section. We have analyzed the changes in the fibrous

periosteum, the 'external osteogenetic layer' or 'cambium layer,' the compact bone, and the cancellous bone during the healing process from the first to the twenty-fourth day following a simple sawcut made under aseptic conditions. We wish to direct attention to the importance of the cambium layer or the osteogenetic layer of the periosteum which lies against the true bony tissue and which we think is continuous with the lining of the Haversian canals and of the cancellous bone. We also noted two types of bone dissolution, one in the presence of osteoclasts, the other in the absence of the osteoclasts. This latter process, for want of a better term, we have designated 'bone dissolution.'

#### TECHNIQUE

The same technique was used in all cases. A longitudinal cut, 10 to 20 mm. in length, was made with a thin circular saw in the shaft of the tibia in its upper half. Rabbits were used in all of the experiments. The longitudinal cut was chosen because no splints are required to support the leg after operation, and the recovery of material for histologic study, especially in the earlier days of the repair process, is facilitated. There were two infected wounds, otherwise the healing process was uncomplicated. The exact age of the rabbits used is not known. At first, rabbits were bought at random, but it was found that in old animals the inorganic matter in bone was present in such large amounts and the organic matter in such small amounts, that upon decalcification the details of bone structure were lost. The entire series of experiments was then repeated upon young adult rabbits with satisfactory results.

At definite intervals from the second to the twenty-fourth day a segment of the tibia including the cut was removed, fixed, decalcified, embedded in celloidin or paraffin, and sectioned. All of the sections photographed were stained with Ehrlich's haematoxylin and aqueous eosin. Other stains were used on sections for comparison and further study.

The simplest method of presentation of the process of repair will be to describe what can be seen in the sections from specimens of successive days and then to try to outline the rôle of the several elements in the process.

### *First day*

At the end of the first day a blood clot fills the cut. In it are bone splinters of varying sizes on which giant cells are already at work.

The cambium shows proliferation with thickening on either side of the cut. The fibrous periosteum is swollen. The endosteum shows little or no change.

There is, then, a very prompt response by both the cambium and the fibrous periosteum expressed in one case by proliferation, in the other case by oedema, which gives a distinct thickening of these layers. All of the proliferative changes are at some distance from the cut. For the most part, this is due to destruction of the cells in and near the cut. Overstimulation of cells may also be a factor.

### *Second day*

At the end of the second day there are a few changes in the clot. There is now a large mass of fibroblasts in its inner part.

The cambium and the fibrous periosteum show a more advanced stage of the conditions mentioned under the first day. The cambium is thickened for a considerable distance on either side of the cut. The fibrous periosteum is from two to three times its original thickness, due to oedema and cell proliferation. The endosteum now also shows proliferative changes. There are, then, at the end of the second day, proliferative changes in the fibrous periosteum, cambium, and endosteum, but there is as yet no new bone.

Figure 1 is a photograph of low magnification, showing the general condition at the end of the second day. It is also illustrative of the type of injury involved. The cut, the clot, and the relations of the proliferative changes to the cut are evident.



Figure 2 is a photograph of higher magnification of the area above the cut in figure 1. It shows the changes in the fibrous periosteum and the cambium in more detail. The cut is seen in the right lower quadrant of the figure.

#### *Third day*

At the end of the third day the sections show no marked changes from the conditions described for the second day.

#### *Fourth day*

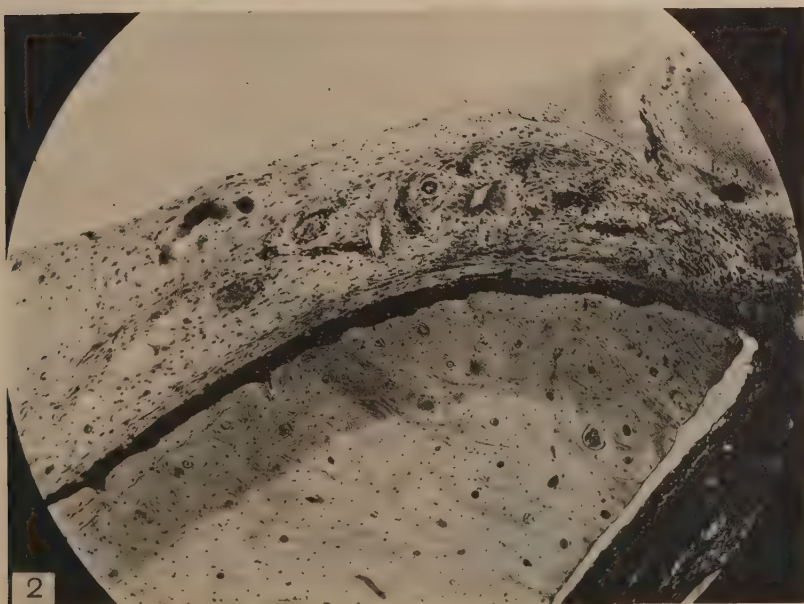
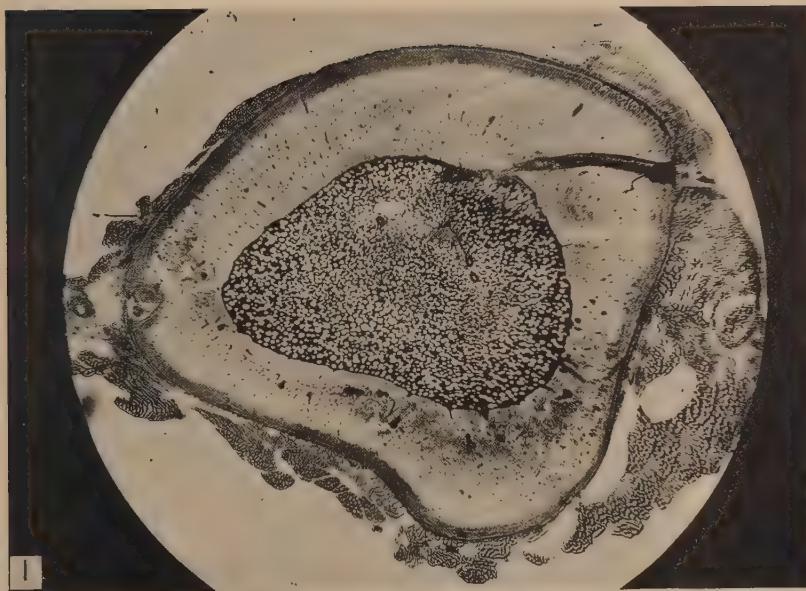
At the end of the fourth day the changes in the cambium, the fibrous periosteum, and the endosteum are accentuated and new bone is present.

The new bone appears as a series of dentations beneath the cambium. This is well shown in figure 3. There is also new endosteal bone. Here the condition is somewhat different. There is a thin layer of new bone from which long slender spicules, covered by a heavy epithelioid endosteum, project into the marrow cavity. A few of these spicules coalesce. These spicules are pictured in figure 4. We now have the beginnings of both an internal and an external bony callus. It will be seen that each plays an important part in closing the wound, and in this series of experiments they are of about equal value.

There is a marked difference in structure between the cambium and the endosteum at this stage. The cambium forms a continuous layer several cells thick with an unbroken outer surface, but with an inner surface that covers the spicules of new bone and fills the interspaces between them (fig. 3). The endosteum, on the contrary, simply follows the contour of the spicules of new bone (fig. 4) and is relatively thin.

#### *Fifth day*

At the end of the fifth day the sections from the two specimens are quite different in that one shows cartilage and the other does not. We will first discuss the bony calluses and then turn to a discussion of the cartilage as a separate problem.



Figs. 1 and 2 In figure 2 the cambium is represented by the dark band just outside the bone. It falls short of the cut as is better shown in figures 1 and 5.

The most interesting advance over the preceding days is the breaking down of the line of demarcation between the external callus and the old cortical bone as a result of absorption of the cortical layer by osteoclasts.

The cambium shows much the same picture as at the end of the fourth day. The external callus is larger and more cancellous. In one specimen it is almost as thick as the compact bone of the shaft; in the other specimen it is much thinner.

The condition of the internal callus is quite different in the two specimens. In the one case it is large and completely bridges the cut. In the other case it is just beginning to form. In the latter specimen there was a larger haemorrhage into the marrow cavity. This may have retarded the formation of the internal callus.

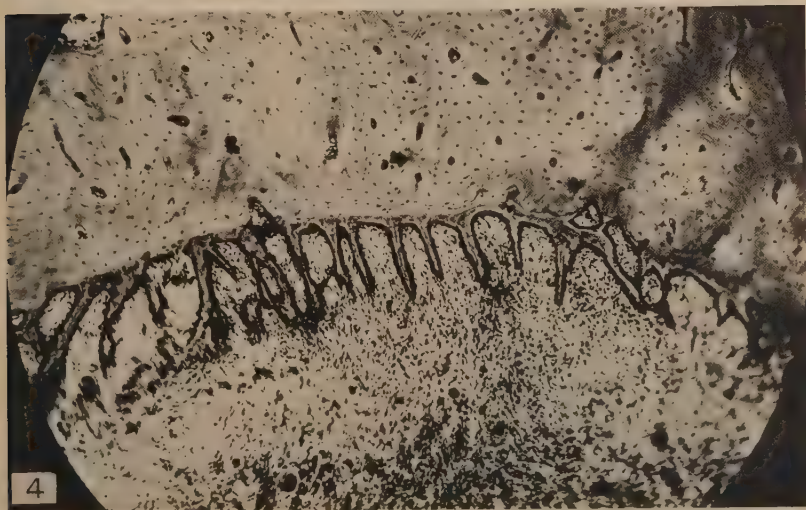
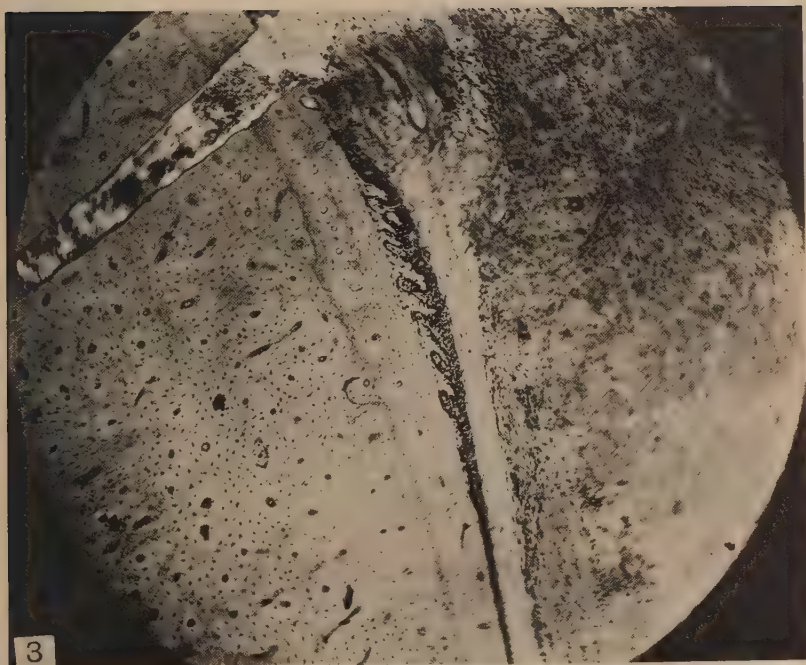
As mentioned above, cartilage is present in one specimen, but not in the other. Throughout our later stages we found it occasionally, but not constantly. For the first time cartilage is present. It is in relation to the external callus on either side of the cut. The general relations are: first the old bone, then the new bone, cambium, fibroblasts, cartilage, and then more fibroblasts. The cartilage, for the most part, is completely surrounded by fibroblasts, but in limited areas the cartilage and the new bone are in contact.

There seems but little doubt that the fibroblasts give rise to the cartilage, but the source of the fibroblasts is less clear. There is some evidence that the cambium has contributed to their formation. There is an intimate relationship between the fibroblasts and the fibrous periosteum, especially its deeper layers. Then there is, of course, the possibility that the fibroblasts have migrated into the region of the cut.

### *Sixth day*

At the end of the sixth day the actual filling in of the cut has begun, in this case largely by the extension of new bone from the internal callus.





Figures 3 and 4



In the specimen at hand the external bony callus is small and cancellous. The cambium is active and fibroblasts are pouring into the outer end of the cut.

The internal bony callus, though small, completely bridges the end of the cut and extends outward into the cut.

Within the cut there is a thin layer of bone along either side. This is in continuity with the internal bony callus and extends throughout the inner four-fifths of the cut. The outer end of the cut and the adjacent areas are filled with fibroblasts.

### *Seventh day*

The essential features of the process of repair on the seventh day are as follows:

- a. Osteoblasts have entered the cut from the marrow cavity.
- b. The external osseous callus has begun to unite with the original bone and has crossed the line of the cut.
- c. The internal osseous callus has bridged the cut.
- d. Two masses of cartilage are present in the external callus.

The cut, which is shown in the lower part of the photograph (fig. 6), contains a relatively large amount of vascular connective tissue, osteoblasts, several giant cells, and at its inner end a mass which appears to be a spicule of new bone. The connective tissue within the cut is continuous with the connective tissue of the external callus; at the inner end of the cut it abuts upon the bone of the internal callus. The osteoblasts within the cut are beginning to line up along its walls, but they have not yet begun to deposit bone. The repair process in the specimen of the sixth day evidently was more rapid than in that of the seventh day because of the presence of new bone within the cut in the former specimen and its absence in the latter. There are several large cells lying close to one of the walls of the cut near its inner end. Occupying the inner one-third of the cut there is a mass of tissue which is quite cellular in nature and which appears to be bone that has been cut near the surface so that many of



Figures 5 and 6

the osteoblasts and little of the bone are present. This mass is continuous with the internal bony callus. It is probably a projection of the bone of the internal callus into the cut.

In the specimen from which the figure is taken there are two masses of cartilage—one, which shows in the figure opposite the outer end of the cut; a second, which does not show in the figure, to the left of the cut and at an appreciable distance from it. The cut edges of the fibrous periosteum flare out in contact with these cartilaginous masses. This flaring is more noticeable in relation with the cartilage to the left of the cut, which does not show in the figure. The mass of cartilage to the left of the cut lies close to the cortex of the original bone, a relatively thin layer of new bone being interposed between the two. There is a considerable amount of new bone in apposition with all of the surface of the cartilage which faces the cut, simulating the formation of a barrier between the cartilage and the cut. There are a few cartilage cells embedded in the substance of this bone. We interpret the significance of cartilage cells in this position as an invasion of the cartilage mass by blood vessels and its replacement by bone. This process is illustrated in figure 8, and will be more fully discussed with the description of that figure. There is a wider area of new bone interposed between the cartilage to the right of the cut and the cortex of the bone. This mass of cartilage extends to and crosses the line of the cut, but is separated from the cut by the interposition of new bone.

The cambium layer is present as a distinctly thickened layer of cells. This is well illustrated in the extreme upper part of the figure. The cells at the cut edge of the cambium flare out in relation with the masses of cartilage and the line of contact between this layer and the fibrous periosteum is obscured.

The external bony callus is relatively thick, more so to the right of the cut than to the left. A distinct line of demarcation is visible between the external bony callus and the cortical bone. The wavy character of this line is indicative of a

slight amount of absorption of the external surface of the original cortical bone having taken place. In localized areas at some distance from the cut, the line is less distinct, projections from the external bony callus extending into the original cortex simulating dovetail union. Osteoclasts are not definitely discernible in the external bony callus.

The internal bony callus is not quite as thick as the external. The internal bony callus has completely bridged the cut and extends approximately halfway around the marrow cavity. Union of the internal bony callus with the cortex of the original bone is complete, there being no clear evidence of a line of demarcation as there is between the external bony callus and the original bone. The internal osteogenetic layer shows evidence of activity, the cells being more or less cuboidal in shape, and in places existing as a double layer. No clearly defined osteoclasts are present in the internal bony callus.

Phagocytes are numerous in the connective tissue opposite the external end of the cut.

#### *Eight and one-half days*

The condition of the fibrous periosteum, cambium, cartilage, external and internal bony calluses at this stage in the repair process is very similar to that as described for the seventh day.

In addition to the presence of the connective tissue within the cut, the osteoblasts have begun to become arranged along the walls of the cut, and are beginning to deposit bone on the surfaces of the walls of the cut. New bone from the callus on one side of the cut is just beginning to enter it. Union between the external bony callus and the cortex of the original bone is more intimate than it is on the seventh day, but in places the line of demarcation between these two elements is still visible. In the internal bony callus there are areas suggestive of beginning bone dissolution. Phagocytes are present in the connective tissue opposite the external end of the cut.



*Ninth day*

The essential steps in advance on the ninth day are:

*a.* The external bony callus has entered the cut.

*b.* Absorption of the external and internal bony calluses by osteoclasts, and of the internal bony callus by bone dissolution.

The cut (fig. 7) contains a large amount of fibroblastic connective tissue. At its inner end there is a spicule of new bone which is continuous with the internal bony callus. The osteoblasts within the cut have deposited a thin layer of bone on the surface of the walls of the cut. This is most marked toward the inner end of the cut. This bone which has been deposited by the osteoblasts within the cut is continuous with the internal bony callus.

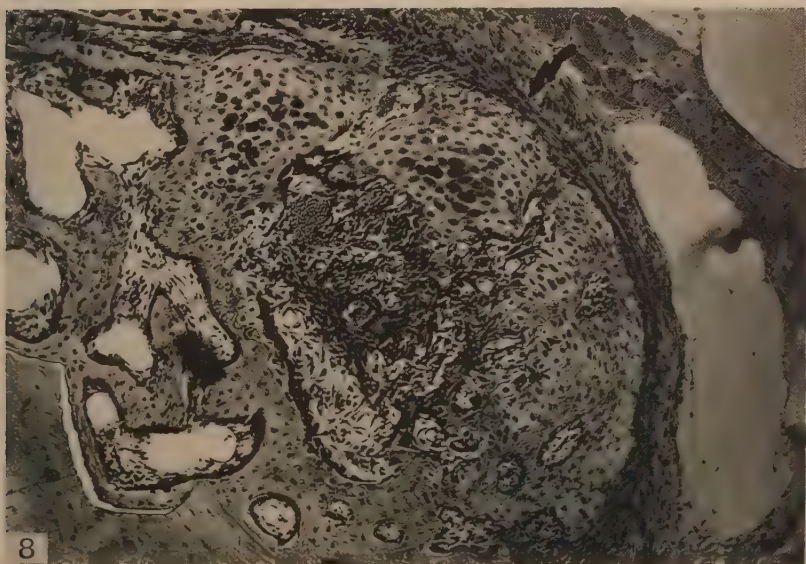
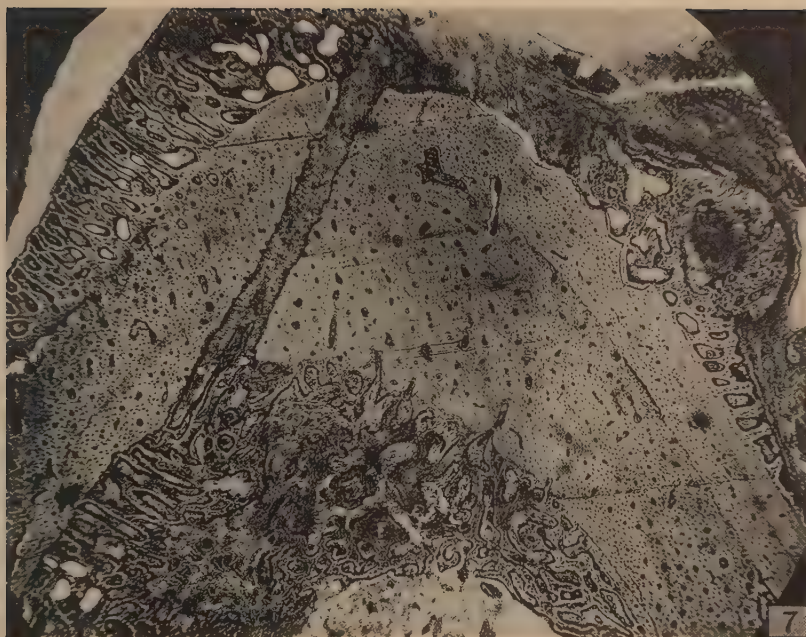
The external bony callus to the left of the cut has crossed, and has begun to enter, the external end of the cut. The external bony callus to the right of the cut falls short of the cut by a considerable distance. This is due to the fact that in the operation the saw removed the cambium layer over this area before it entered the cortical bone.

In the external bony callus to the left of the cut there are two cartilage cells which are completely surrounded by bone. In the external bony callus to the right of the cut there is a mass of cartilage. Figure 8 is a higher magnification of this area. This mass of cartilage is being invaded by blood vessels and is completely surrounded by new bone. The fibrous periosteum has grown over its outer surface.

The bone of the external and internal calluses contains many osteoclasts which are enlarging the canals by absorption of the new bone.

There are small areas of bone dissolution in the internal bony callus. A discussion of this process is given in the description of a subsequent figure.

Phagocytes are still present in the connective tissue opposite the external end of the cut.



Figures 7 and 8

*Tenth day*

In one of the specimens of the tenth day there are two elements which present differences in structure from those of preceding days, namely, the external bony callus and the cartilage within it.

The external bony callus has completely bridged the cut. The bone within the cut connects the external and the internal bony calluses.

In one of the sections the condition of the cartilage is much the same as on the ninth day, except that isolated cartilage cells embedded in new bone are present at the external end of the cut. In another section there is a large mass of cartilage at a considerable distance from the external end of the cut. The condition in this case differs from that of earlier days in that the fibrous periosteum and the cambium layer are present between the lateral two-thirds of this mass of cartilage and the cortex of the original bone. There is a large amount of new bone between all of the mass of cartilage and the original cortex.

*Twelfth day*

The osteoblasts within the cut have deposited a considerable amount of bone which is cancellous in nature and which is continuous with the external and internal bony calluses. There is a considerable degree of absorption of the original bone along the walls of the cut, especially at the external end of the cut.

The external and internal bony calluses have become reduced in size. A few osteoclasts are present in the external bony callus, but cartilage is absent. Bone is being deposited in the large canals toward the periphery of the bone.

The fibrous periosteum has united across the external end of the cut, and the cambium layer has almost done so. Osteoclasts are present between the cambium layer and the bone even at a considerable distance from the cut.





Figures 9 and 10



*Thirteenth day*

The reparative process is not as far advanced in this specimen as in that of the twelfth day. The two layers of new bone within the cut have become connected (fig. 10). There is a slight degree of separation between the new bone and the walls of the cut due to the process of fixation. Absorption of the original bone along the walls of the cut is not as extensive as it is on the twelfth day, but there are two areas on the left wall of the cut where absorption is noticeable. Except for these two areas, the walls of the cut are uniformly straight.

The external bony callus has not become reduced to the extent that it has on the twelfth day, but there is a considerable amount of absorption of the new bone. The external bony callus contains two masses of cartilage which are being replaced by bone. The fibrous periosteum has united across the line of the cut.

The internal bony callus is small in amount and contains definite areas of bone dissolution, especially at the inner end of the cut. There are few if any osteoblasts on the marrow side of the internal bony callus.

*Fifteenth day*

The status of repair on the fifteenth day (fig. 11) is much the same as that for the thirteenth day, except that, *a*) the cut is completely filled with bone; *b*) the internal callus is considerably larger and is undergoing marked bone dissolution; *c*) the external bony callus has securely bridged the cut; *d*) there is no evidence of a cartilaginous callus.

In figure 11 it will be noted that the cut is completely filled with new bone. The channels in this new bone are smaller than in preceding sections, due to the increased amount of calcareous deposit. At intervals, however, the channels have been extended by the process of osteoclasia through the laminae of new bone lining the old and into the old bone itself. This is an important process, as will be seen from later sections, in establishing a firm union.

In the external callus the process of note is that of excavation of the new bone and the extension of this process into the old bone. This is especially conspicuous to the left of the cut (fig. 11). Osteoclasts are active not only in these enlarged channels, but also under the cambium covering the bony callus. This reduction of the external callus takes place all along the surface of the bony callus, except opposite the external end of the cut where osteoblasts are still actively laying down new bone. The osteogenetic cells of the cambium, except those in the region over the external end of the cut, show signs of regression. They are few in number and are atrophic. There is no cartilaginous callus.

In the section pictured in figure 11 there is a large internal callus. This does not mean that it has increased in size over those of preceding days; as a matter of fact, it has already undergone reduction, as can be established from the structure of the marrow adjacent to the callus. The internal callus in this case is large, but it had probably reached its maximum development on about the ninth or tenth day. The callus is thickest opposite the internal end of the cut, which is markedly different from that of the external callus. Reduction of the internal callus is well under way. Its removal is effected by two methods already referred to, osteoclasia and bone dissolution. The reduction by osteoclasia is very marked in this case, more so than in other specimens. Osteoclasts are very active, especially on the marrow side of the spicules of the callus. The process of dissolution begins at the center of the spicules and especially in the spicules around which there are few or no osteoclasts. The process is at first recognized by the deeper blue stain which the center of the spicule takes when stained with haematoxylin and eosin. The cytoplasm next swells and the nucleus disintegrates, leaving a space. The matrix around it becomes granular and vacuolated, giving the appearance of fat necrosis (fig. 12). We have not determined the chemical nature of this process, but the color reaction obtained with haematoxylin leads us to believe that it is a mucoid degeneration. This

process usually begins at a time when osteogenetic activity is still going on at the periphery of the spicule.

Fibroblasts are still quite embryonic over the callus in the region of the cut. They lie parallel with the surface of the bone, thus indicating the direction of the future periosteal fibers.

In the connective tissue opposite the external end of the cut there are still a number of phagocytes. Their fate is not indicated.

#### *Sixteenth day*

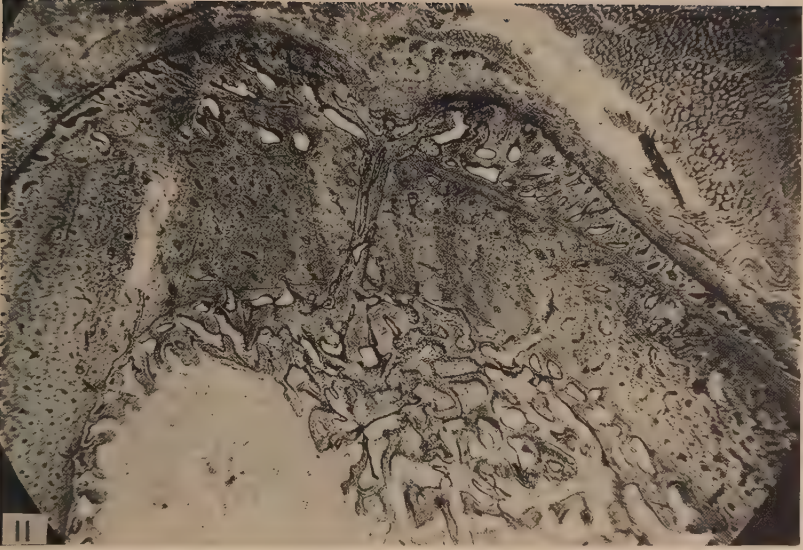
The conspicuous contributions of the material of the sixteenth day are: *a*) the reduced external and internal bony calluses; *b*) increased callus formation opposite the external end of the cut; and, *c*) extended resorption between new and old bone.

The external bony callus is much reduced except over the external end of the cut, where it has attained the same thickness as the rest of the callus. The callus is rapidly retreating before the army of osteoclasts which have formed a line immediately beneath the cambium. Large spaces have also been excavated between the callus and the old bone. There is no cartilaginous callus.

The internal bony callus is considerably reduced, but shows little else that is new.

#### *Seventeenth day*

The material of the seventeenth day presents two important processes: 1) Absorption of bone adjacent to the cut to such an extent that the cut is beginning to lose its identity. The cavities which are formed adjacent to the cut have a narrow lumen next to the cut, but widen out as they extend into the old bone (fig. 13). This is an important process, because these cavities are filled with new bone during succeeding days. Because of the shape of these excavated cavities, the bone which is to fill them establishes a firm dovetail union between the new bone in the cut and the old bone.



Figures 11 and 12



2) The osteogenetic activity of the osteoblasts lining many of these excavated cavities in depositing new bone. Both the external and the internal calluses are being reduced by the methods described. There is also considerable excavation into the old bone between it and both the external and the internal calluses. There is no cartilaginous callus. In figure 13, *a* indicates the region of the cut; *b* indicates an excavation in communication with the cut, and *c* indicates osteoblasts within the excavation.

#### *Eighteenth day*

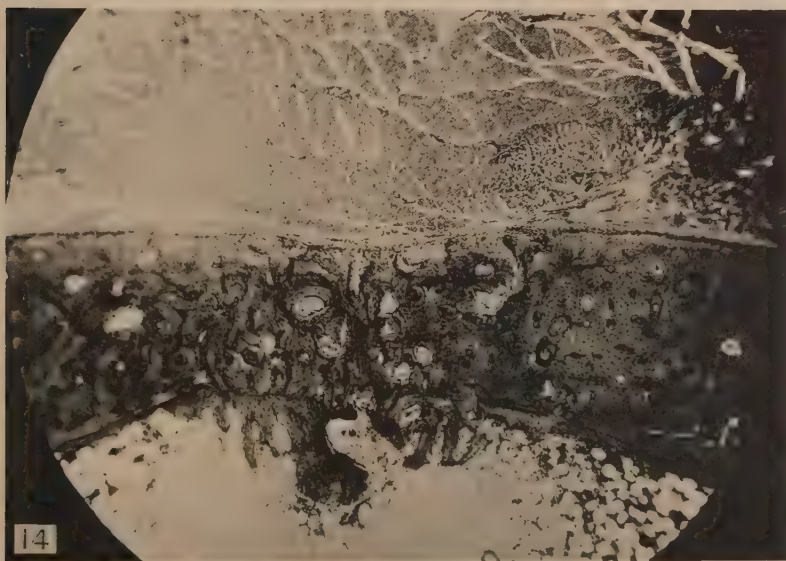
The external and internal calluses are almost completely absorbed. The excavated cavities are being rapidly filled with new bone.

The cut is completely filled with new bone, and the excavated cavities extending from the Haversian systems in the bone of the cut into the adjacent old bone are being rapidly filled with bone. This newly formed bone is laid down in the form of Haversian systems. This process establishes a firm dovetail union between the old bone and the new bone within the cut. While this process of filling is going on other cavities are being excavated.

The external bony callus is almost completely removed. Osteoclasts appear in large numbers on the surface of the remaining external bony callus immediately under the greatly reduced cambium. In some places farther removed from the cut the osteoclasts lie between the cambium and the old bone. These were associated with the absorption of the callus which was at first interposed. There is no indication of a cartilaginous callus having been present. The internal callus, too, is almost completely removed. Osteoclasts are very active on the marrow side of the callus. The periosteum is completely united over the external end of the cut.

#### *Nineteenth day*

The preparations of the nineteenth day contribute nothing new.



Figures 13 and 14

*Twentieth day*

Our only twentieth-day specimen is atypical. The cut has the appearance of a thirteen-day stage. The internal callus looks like that of a fifteen-day or seventeen-day stage. It was, however, an unusually large callus, half filling the marrow cavity. Bone dissolution is very extensive in it, and half of the callus is being removed by this process. The external callus is well reduced, but apparently it never was very large.

*Twenty-first day*

Figure 14 is a typical picture of the status of repair at this stage. The external bony callus has been entirely removed. The process of removal has also included the surface of the original bone under the callus, so that the entire plate of bone in the region of the cut has been considerably thinned. In addition to this thinning there is considerable excavation into the old bone adjacent to the cut. Some of the excavated cavities have already been filled with new Haversian systems, while other cavities are still being formed. The bone in the region of the cut is so excavated and partly refilled with Haversian systems that the outlines of the cut are almost obliterated.

In figure 14 it will be noted that the internal callus is small. In another specimen at this stage the internal callus has been completely reduced. When the internal callus is present, large numbers of osteoclasts are active on its marrow side. A marked process of bone dissolution is clearly apparent in the main mass of the callus.

Giant cells are still present in the connective tissue surrounding the muscle fibers outside the well-formed periosteum.

*Twenty-second day*

We have no record of the twenty-second-day stage.

*Twenty-third day*

This stage presents appearances much the same as those described for the twenty-first day. The old bone adjacent to the cut is markedly excavated, so that the margins of the cut are almost obliterated. The external bony callus appears to have been very large, but at this stage it has been completely reduced for some distance to either side of the cut. Even the old bone is reduced in this region. Still farther removed from the cut, some of the external bony callus is present.

The internal callus has almost disappeared. Osteoclasts are still active on the marrow side of the callus. Bone dissolution is here seen in its last stages. In this area there are but few reticulated nuclei. The entire area is finely granular, with many small and large round droplets. The main mass has a bluish color. Adjacent to the internal callus are many fine, long fibroblasts. Between the fibroblasts are many of the clear droplets just mentioned.

*Twenty-fourth day*

Our twenty-fourth-day stage adds nothing new. Our series does not include the complete description of the process of repair, but the later stages, which are not included, can be foreseen. There remains to be accomplished only the filling in of the excavated areas with new Haversian systems.

## DISCUSSION AND SUMMARY

The essential structures involved in the process of repair, as indicated in this series of experiments, are the osteogenetic cells lining the marrow surface of the bone, those lining the Haversian canals, and those constituting the cambium layer. The cambium layer exhibits the most marked changes at the beginning of the repair process. The other osteogenetic cells mentioned are inward continuations of this cambium layer. On the second day after operation, the cambium on either side of the cut becomes prominent by enlargement and mul-



tiplication of its cells. At first, the cells of the single squamous-like layer become cuboidal. Next, a stratified cambium is formed. Often a cambium of six or more cells in thickness is encountered. Spicules of bone are next formed in it on the underlying bone. The cambium thickens in this manner for a distance of one-eighth to one-fourth the circumference of the bone on each side of the cut. In many of our preparations the cambium was injured or scraped by the saw in making the cut. Such injured cambium did not develop and the callus was formed only from the uninjured cambium (fig. 7). The bony callus is very rapidly formed from this proliferated cambium between the fourth and fifteenth days. Over the external end of the cut the process continues until the eighteenth day. The enlarged cambium farthest removed from the cut, however, begins to become reduced as early as the ninth day when osteoclasts begin to absorb the newly formed callus immediately beneath it. This reduction of the cambium and the absorption of the callus gradually continue toward the cut until about the eighteenth or nineteenth day, when they take place all over the callus.

That the deepest layer of the periosteum is concerned in the repair of bone has been described as early as 1742 by Duhamel, who compared it to the cambium in plants. There has, however, existed much controversy whether or not the periosteum is concerned in repair. Ollier (1867) removed periosteum and transplanted it, and concluded from his observations that regeneration of the transplanted periosteum would occur only when the deep, cellular layer was present. Our observations confirm these early findings. The periosteum strips very easily, leaving the cellular layer or cambium in contact with the bone. Such a periosteum will not regenerate bone directly, but may produce cartilage which then changes to bone. If, however, the periosteum is removed by scraping along the bone so as to remove the cambium with it, then regeneration will occur directly from the osteogenetic cells of the cambium.

The significance of the cartilaginous masses in our preparations is not clear. Large cartilaginous calluses, such as are described in the literature as existing in fracture healing, are not present in our preparations. Wieder describes such cartilaginous calluses in his experiments on complete fractures, but reports absence of cartilage in the healing of saw-cut wounds. The cartilage masses in our preparations are not large and are always developed independently of the bony callus. The cartilage masses are always found in the young connective tissue at the cut ends of the fibrous periosteum outside the cambium. They never bridge the cut in our preparations. About the seventh to ninth days osteogenetic buds from the underlying bony callus invade them and change them to bone, after which they are rapidly reduced.

There are two methods by which the bony calluses are reduced. Osteoclasts is a well-known process of bone absorption and requires no further discussion except that it is practically the sole method by which the external callus is removed and the excavations into the old bone are accomplished. In a few instances bone dissolution was noted in large external calluses. In the removal of the internal callus the osteoclasts play a part; in cases, however, where the internal callus is large, a process of bone destruction takes place, which process we have defined and described as 'bone dissolution' in our description of the fifteenth-day stage. This dissolution, which has the appearances of both fatty and mucoid degenerations, is noted as early as the ninth day. It usually takes place at the center of the bone spicules at a time when osteogenetic cells still appear active on their surfaces. Since this dissolution occurs in large calluses where osteogenesis is still going on, a vascular or nutritive disturbance at the center of the spicules may possibly be the initiating cause of this process.

## CONCLUSIONS

This study has presented the following evidence:

1. The cambium layer, the osteogenetic cells lining the Haversian canals, and the endosteum are all active in the production of new bone to form the calluses and to reunite the old bone at the line of fracture.
2. The union of the severed bone is strengthened by means of a sort of dovetail union made possible by the extension of the new bone forming in the cut into the excavated spaces of the adjacent old bone.
3. Reduction of the external bony callus is accomplished by osteoclasia and that of the internal bony callus by bone dissolution and osteoclasia.
4. Cartilage when present arises from connective-tissue cells at the cut ends of the fibrous periosteum.

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## A METHOD OF BLOOD STAINING WHICH REPLACES INJECTIONS

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ONE FIGURE

The difficulties of following the distribution of blood vessels in organs by injection, which is especially noticeable in smaller embryos, led me to search for another method which would give results similar to satisfactory injections. Rost ('10-'11) made the blood capillaries visible by staining the nuclei of the blood cells in the vessels. He first poisoned tadpoles with hydroxylamin or arsine, destroying the plasma of the corpuscles, and then stained the residual nuclei with methylen blue, thionin, or other dyes. His pictures show the capillaries clearly, but there is also staining of the nuclei in the epithelial and connective-tissue cells. Moreover, it is evident that his method is useful only in those animals possessing nucleated erythrocytes and cannot be employed in mammals.

I have developed another staining method by which the blood corpuscles are strikingly differentiated from the other tissues. This method gives results in embryos and is not difficult. It is only necessary that the corpuscles be well fixed. If they lose their hemoglobin during fixation, as often happens, then the method will fail. The most important factor is, therefore, the fixation, and here care must be taken that the vessels remain filled with blood.

For fixation, formol in the ordinary 10 per cent solution gives very good results. It is necessary to cut the organ into

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relatively thin pieces or slices. This is done in the formol solution so that fixation occurs immediately and blood loss is prevented. Good results can also be obtained with Regaud's solution (80 cc. of a 3.5 per cent solution of potassium bichromate and 20 cc. of concentrated formol) and other fixatives.

Celloidin embedding is preferable, because it prevents the corpuscles from being washed out and because thicker sections can be obtained. Such preparations should be thick in order that the capillaries can be followed to a greater extent. This can be done by my method in which sections up to 90  $\mu$  thick are stainable.

The staining itself is easy. The sections are first stained with carmin. For this purpose my new copper carmin can be recommended, since it gives results which are superior to any other carmin stain. To 100 cc. of a 2 per cent solution of neutral acetate of copper (the same as is used in the original Weigert method) are added 2 grams of carmin; the mixture is well ground in a mortar in order to get a fine emulsion. The carmin will of course sink to the bottom, which does not matter. This is only the 'stock mixture.' It is stable and keeps for a long time. Before use it must be well shaken, and then the necessary small quantity is poured in a glass. To this is added ammonium hydroxide drop by drop until the carmin is completely dissolved. Solution is detected by putting a drop of the liquid on filter-paper. If there is any undissolved carmin, grains will be visible on the paper; but if the carmin is all dissolved, there will be a uniform spot from the center to the edge.

The stain is now ready and must be used immediately. The staining takes fifteen to twenty minutes. It is also possible to use a solution of only 0.5 per cent carmin in 2 per cent copper solution. In this case the sections can be left for eighteen to twenty-four hours in the stain. After staining it is necessary to differentiate in acid alcohol. I use 5 drops of concentrated hydrochloric acid in 100 cc. of 70 per cent alcohol. In this process differentiation occurs and the celloidin, which often is deeply colored, becomes decolorized.

The sections, after being rinsed in distilled water, are put in a solution of indigocarmin (this should be called soluble indigo, for it has nothing in common with carmin) in distilled water. The quantity of the stain is not important; it can



Fig. 1 Section of cat liver stained according to method described in text. Blood vessels are black.

be about 1 per cent. Sometimes it is better to add a few drops of acetic acid to the color, but that is not always necessary. The sections are left some minutes in the indigocarmin, but they can remain there overnight. Afterward they are rinsed, and are then a uniform blue. They must be further

differentiated in a saturated aqueous solution of picric acid. This procedure takes some seconds and can be controlled with the microscope.

If the blood was well fixed, the sections will be now quite as good as injections, as can be seen from the photograph. Sometimes the connective tissue has a tendency to retain the blue stain, but this can be remedied by using for the terminal differentiation not pure picric acid, but van Gieson's picrofuchsin. The acid fuchsin has a greater affinity for the connective tissue and will mask the indigo so that only red blood corpuscles will be stained blue.

The mounting of the sections is done in the ordinary manner and the preparations keep a long time without fading.

A further advantage of this method is that the organs are not spoiled for study by other histological methods as they are in the method of Rost or by injection, and this is particularly important in embryos.

The procedure can be summarized as follows:

1. Staining of the sections in carmin.
2. Rinsing in distilled water.
3. Treating with acid alcohol.
4. Rinsing in distilled water.
5. Staining with indigocarmin.
6. Rinsing in distilled water.
7. Differentiation in picric acid or in picrofuchsin of van Gieson.
8. Treating with 95 per cent alcohol.
9. Clearing in origanum or some other oil.
10. Mounting in Canada balsam.

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THE AMERICAN MEDICAL DICTIONARY, by W. A. Newman Dorland, thirteenth edition, revised and enlarged (pronunciation, derivation, and definition); 1925, illustrated in black and colors. Size  $5\frac{1}{4} \times 9$ , flexible, patent thumb alphabetical lettering for easy reference, \$7.50, W. B. Saunders, Philadelphia. All sections of Medicine and the applied sciences have received attention. About 2500 new words have been added. In addition to the usual anatomic and clinical tables, there are specially prepared tables of tests, staining and staining methods, methods of treatment, etc. Especially valuable for clearness and accuracy is the use of slightly heavier-faced type for the words than for definitions and the carefully discriminating employment of capital letters. The book contains 1344 pages and weighs only 3 pounds 4 ounces.

THE VERTEBRATE SKELETON FROM THE DEVELOPMENTAL STANDPOINT, by J. S. Kingsley; 1925, 338 pages, 324 illustrations in line and halftone, and a word index of 12 pages, size  $6 \times 9$ , cloth, \$6.00, P. Blakiston's Son & Co., Philadelphia. *From the Preface:* "This volume aims to give an outline of vertebrate osteology, tracing the skeleton elements from their early appearance to the adult condition, for only by ontogeny can homologies (or their lack) be ascertained in the various groups. Since knowledge of the adult structure of higher vertebrates is best obtained by comparisons with lower forms, greater stress has been laid on Ichthyopsida and on reptiles than upon birds and mammals, which have been more adequately treated elsewhere than have lower groups. There is also reference to extinct forms, as these frequently throw light upon living species. The work is intentionally descriptive and no attempts have been made to trace lines of descent, although here and there hints have been given of the relation of groups." A very complete and useful bibliography of 11 pages contains 796 classified references.

THE OLD AMERICANS, by Aleš Hrdlička; 1925, 438 pages, 38 portrait illustrations. Size  $6\frac{1}{2} \times 9\frac{1}{2}$  uncut, cloth, \$10.00, The Williams & Wilkins Company, Baltimore. The seven chapters are: 1, Introduction. 2, Historical sketch of the old American stock. 3, Pigmentation. 4, Measurements and morphological observations (stature, weight, sitting height, arms, head, and head shape, mean height index, size of head, face, facial constituents, ears, chest, hand, foot, leg). 5, Physiological observations (pulse, respiration, temperature, muscular strength). 6, Abstracts. 7, The future American type. The index occupies 26 pages.

ANIMAL CLASSIFICATION AND DISTRIBUTION, a reference book for students of elementary zoölogy at secondary schools, colleges, and the universities, by Douglas M. Reid, with tables, zoogeographical map, and diagrams; 1925, 52 pages. Size  $5\frac{1}{4} \times 7\frac{1}{4}$ , stiff cloth, Charles Griffin & Company, Limited, London, and J. B. Lippincott Company, Philadelphia. It is a time-saving synopsis with provision for a digest of lecture notes and sketches, and a reference book showing the modern animal classification. The Phyla, classes, and orders are grouped in sections for easy reference and are self-explanatory. The tables help to indicate



## BOOKS AND PUBLICATIONS

the alternate classification past and present, and are so arranged as to show at a glance how they correspond. The zoogeographical map should also prove useful. The typographical arrangement and spacing, together with a very careful and discriminating use of italic and heavy-face type, add greatly to the clearness of the text and ease of reference. The printing is on the odd pages, leaving blank the even pages for notes that the student may desire to enter for his own permanent reference.

**ABSTRACTS OF THESES**, The University of Chicago Science Series (1922-1923); 1925, 500 pages. Size  $6 \times 9$ , cloth, volume I. It presents the essence of the contributions to knowledge made by candidates for the doctorate in the Ogden Graduate School of Science, and contains papers by 103 authors, in thirteen departments—mathematics, astronomy and astrophysics, physics, chemistry, geology and paleontology, geography, zoölogy, anatomy, physiology, botany, pathology, hygiene and bacteriology, home economics. The preface states that "the abstracts are to be published in two series: the 'Humanistic Series,' including the abstracts of theses in the Graduate School of Art and Literature, the Divinity School, the School of Commerce and Administration, and the Graduate School of Social Service Administration; and the 'Science Series' containing the abstracts in the departments of the Ogden Graduate School of Science. One volume in each series is to be published annually.

**AN INTRODUCTION TO THE FINER ANATOMY OF THE CENTRAL NERVOUS SYSTEM, BASED UPON THAT OF THE ALBINO RAT**, by E. Horne Craigie; 1925, 194 pages, 38 line and halftone illustrations. Size  $5\frac{1}{4} \times 8$ , cloth, \$3.00. Publishers, University of Toronto Press, Toronto, and P. Blakiston's Son & Co., Philadelphia, Pa. The bibliography of ten pages gives 89 references. The appendix treats of the preparation of sections—method of Weigert. The preface states that the book is an attempt to fill a gap, caused by the lack of a "small handbook devoted to the minute anatomy of the nerve centers and pathways—which is at least as important as the gross anatomy—in any laboratory animal," . . . and "to provide a brief but comprehensive introduction to the functional anatomy of the central nervous system of a common laboratory type, of size convenient for the use of serial sections."

The book is designed primarily for elementary students.

**DIRECTIONS FOR THE DISSECTION OF THE CAT**, by Robert Payne Bigelow; 1925, 48 pages. Size  $5 \times 7\frac{1}{2}$ , cloth. The Macmillan Company, New York. *Preface*: In its essential features the body of the cat is very similar to the human body. This fact, together with the abundance of the material and the convenient size of the animal, makes the study of the anatomy of the cat a desirable prerequisite for college courses in human physiology and personal hygiene, and a valuable part of a system of premedical education . . . The aim is to guide the student so that he may obtain by direct observation a clear vision of the organism as a whole, not as a series of isolated systems of organs. For this reason the program of dissection is arranged so that it may be carried out on a single specimen.

**HUMAN PHYSIOLOGY**, by William Furneaux (revised by William A. M. Smart); 1924, 290 pages, 219 illustrations, size  $5 \times 7\frac{1}{4}$ , cloth, \$1.30, Longmans, Green & Co., New York. "The general plan of the book and the order of the chapters have been retained, but otherwise it has been almost completely rewritten. Being an essentially elementary book, it must include some of the simpler facts of anatomy for the proper elucidation of the subject."—*Preface*.